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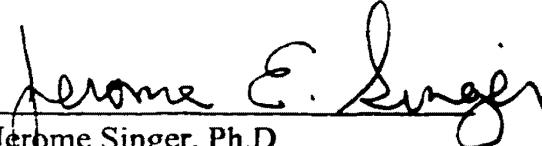
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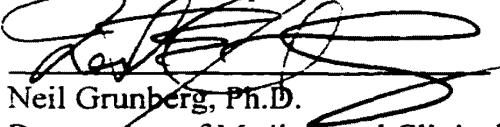
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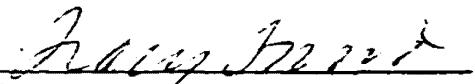
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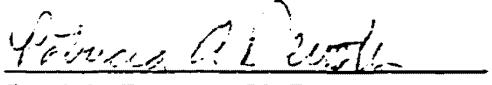
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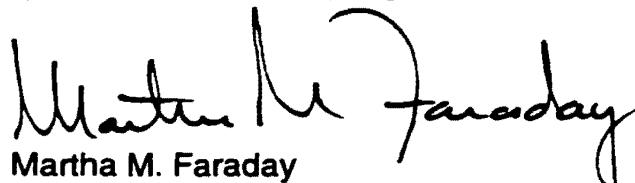
  
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## **ABSTRACT**

Title of Thesis: **"The Role of Sex and Strain in Behavioral and Biologic Stress Responses of Rats"**

Author: **Martha M. Faraday, Doctor of Philosophy, 2000**

Thesis directed by: **Neil E. Grunberg, Ph.D.**

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Stress has been implicated in the etiology of many behavioral disorders (i.e., drug abuse, feeding disorders) and disease states (i.e., hypertension, diabetes, depression). Individuals differ, however, in vulnerability to stress-related disease. The goal of this doctoral research was to identify potential behavioral and possibly biochemical markers of stress vulnerability vs. resilience in male and female rats of two strains (Sprague-Dawley and Long-Evans) that might predict eventual development of specific stress-related behavioral disorders or diseases in certain subgroups of humans. The experiment assessed the effects of mild, repeated daily stress on multiple behaviors and biochemical indices within the same subjects to construct a detailed model of potential markers of stress vulnerability vs. resilience.

Specifically, subjects were exposed to no stress or to 20 min/day immobilization stress for three weeks. During this period, behaviors were measured in two domains: 1) body weight, feeding, and locomotion; and 2) acoustic startle reflex and pre-pulse inhibition, passive avoidance, and Morris

water maze performance (three measures of cognitive performance). Hormones of the hypothalamo-pituitary-adrenocortical (HPA) axis were measured at the end of the experiment.

The major findings were: 1) the four subgroups (i.e., two strains of male and female rats) manifested behavioral stress responses that varied with the domain assessed (i.e., feeding, body weight, and activity vs. cognitive performance) and imply different stress vulnerabilities; 2) the four subgroups manifested consistent stress responses within each behavioral domain, suggesting that stress vulnerability may be domain-specific; and 3) stressed animals within each of the four subgroups manifested changes in HPA axis hormones consistent with a stress response. Therefore, behaviors, rather than HPA axis hormones, most clearly differentiated apparent subgroup vulnerabilities. In addition, the findings suggest that certain behaviors (i.e., feeding, acoustic startle and pre-pulse inhibition) may have utility as behavioral markers for domain-specific types of stress vulnerability in humans.

**The Role of Sex and Strain  
in Behavioral and Biologic Stress Responses of Rats**

**by**

**Martha M. Faraday**

**Doctoral Dissertation submitted to the Faculty of the  
Department of Medical and Clinical Psychology  
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## ACKNOWLEDGMENTS

Here then at home, by no more storms distrest,  
Folding laborious hands we sit, wings furled;  
Here in close perfume lies the rose-leaf curled,  
Here the sun stands and knows not east nor west,  
Here no tide runs; we have come last and best,  
From the wide zone in dizzying circles hurled  
To that still centre where the spinning world  
Sleeps on its axis, to the heart of rest.

D. Sayers, 1936

I have come home. Four people showed me the way. Neil E. Grunberg showed me that there was a road that led from the wilderness to here. Peter M. Scheufele walked with me every step of that road. Robert Wiley gave me a lantern to carry — “you are a teacher” — and relit it until it stayed lit on its own. John W. Mason met me at a crossroads and walked with me along the part of the route that he knew. Without each of them, I still would be wandering. Thank you.

Every member of the Grunberg lab — Nate Apatov, Casey Skvorc, Hirsch Davis, Jeff Cook, Bonnie Yatko, Stephanie Nespor, and Brenda Elliott — helped me to run this dissertation. Steph and Brenda were invaluable. Brenda was my right hand. Laura Klein and Matthew Rahman gave me long distance encouragement and advice. Thank you.

Each member of my dissertation committee — Jerome E. Singer, Tracy Sbrocco, Neil E. Grunberg, and Patricia M. Deuster — contributed meaningfully to this doctoral work. The work is stronger and clearer because of their honest criticism and helpful suggestions. Thank you.

*This work is dedicated to  
my parents, Richard and Paula Faraday,  
and to Dennis Seidel,  
for his kindness to animals.*

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Figure 73. Morris water maze distances on Trial 2 of Day 5.

Figure 74. Morris water maze time on Trial 1 of Day 6.

Figure 75. Morris water maze distances on Trial 1 of Day 6.

Figure 76. Morris water maze time on Trial 2 of Day 6.

Figure 77. Morris water maze distances on Trial 2 of Day 6.

Figure 78. Morris water maze time on Trial 1 of Day 7.

Figure 79. Morris water maze distances on Trial 1 of Day 7.

Figure 80. Morris water maze time on Trial 2 of Day 7.

Figure 81. Morris water maze distances on Trial 2 of Day 7.

Figure 82. Morris water maze Trial 1 times of males.

Figure 83. Morris water maze Trial 1 distances of males.

Figure 84. Morris water maze Trial 1 times of females.

Figure 85. Morris water maze Trial 2 distances of females.

Figure 86. Morris water maze average Trial 1 distances over Days 3 to 7.

Figure 87. Morris water maze Trial 2 times of males.

Figure 88. Morris water maze Trial 2 distances of males.

Figure 89. Morris water maze Trial 2 times of females.

Figure 90. Morris water maze Trial 2 distances of females.

Figure 91. Morris water maze average Trial 2 distances over Days 3 to 7.

Figure 92. Corticosterone levels of animals sacrificed on Stress Day 11.

Figure 93. Adrenocorticotropin levels of animals sacrificed on Stress Day 11.

Figure 94. Corticotropin-releasing factor levels of animals sacrificed on Stress Day 11.

Figure 95. Corticosterone levels of animals sacrificed on Stress Day 21.

Figure 96. Adrenocorticotropin levels of animals sacrificed on Stress Day 21.

Figure 97. Corticotropin-releasing factor levels of animals sacrificed on Stress Day 21.

## INTRODUCTION

### Overview

It has been recognized for more than a century that organisms within the same species differ in their behavioral and biologic responses to a given stressor (e.g., Cannon, 1898; Broadhurst, 1960; Mason, 1968a-e; Acri, 1994; Petrides et al., 1994, 1997; Lupien et al., 1997). The potential importance of these individual differences in stress reactivity in terms of physical and psychological health and illness has been demonstrated over the last forty years (e.g., Friedman & Rosenman, 1959; Krantz & Durel, 1983; Suzuki, George, & Meisch, 1988; Lupien et al., 1995; McEwen, 1998). The differential manifestation of stress-induced disease across individuals (e.g., in males vs. females, in individuals of different ethnicities, and in rats of different strains) suggests that, to some extent, vulnerability to stress is biologically-based and conferred by genotype, broadly construed (e.g., Krantz & Durel, 1983; Lemer & Kannel, 1986; Manuck, Kaplan, & Matthews, 1986; Henry et al., 1993; Baum, Gatchel, & Krantz, 1997). Further, studies using rats — in which, presumably, the psychological factors that might produce individual differences have been removed — have demonstrated that biologic and behavioral reactivity to stressors is positively and causally linked to propensity to self-administer drugs of abuse, to development of certain types of physical illness, and to cognitive deficits in old age (e.g., Issa, Rowe, Gauthier, & Meaney, 1990; Sternberg et al., 1989, 1993; Henry et al., 1993; Suzuki et al., 1988).

Despite this body of literature, several important questions remain

unanswered. First, most of the work done in rats has focused on extreme stress phenotypes — rat strains specifically bred for stress sensitivity or stress insensitivity on a particular dependent measure (e.g., Broadhurst, 1960; Driscoll & Battig, 1982; Sternberg et al., 1989, 1992). This focus on two tails of the stress vulnerability distribution has produced a detailed picture of the mechanisms and consequences of extreme stress sensitivity and insensitivity, but has left the continuum between the two extremes relatively unexamined. This omission is important because stress has been implicated in serious illnesses that affect approximately 20 million Americans — about 10% of the population — at a cost of at least 75 billion dollars annually (Murphy, 1996; Hughes, Pearson, & Reinhart, 1984). Further, a recent nationwide survey revealed that almost half of Americans reported using therapies such as stress management to help manage a variety of stress-sensitive but less serious medical conditions, such as arthritis, fatigue, and high blood pressure (Eisenberg et al., 1998). This large number of individuals affected by stress indicates that more than the tails of the stress vulnerability distribution need to be examined. That is, if only extreme stress phenotypes were linked with illness, then one would expect that only about 5% of the population would be affected (based on the assumption that the underlying distribution is normal and the 2.5% of individuals in each tail deviate enough from the distribution's central tendency to constitute a separate population).

Second, this literature has focused largely on the responses of male animals, and left the stress responses of female rats mostly unevaluated. This

omission also is of potential importance because large gender differences exist in human stress-related physical and psychological illness (e.g., Verbrugge, 1985; Lerner & Kannel, 1986; Andersen, 1990; Rapee & Barlow, 1993), indicating that sex is an important variable in certain types of stress vulnerability. In addition, although gender may be a risk factor for certain types of illness, it is clear that gradations of vulnerability exist within each gender (i.e., not every man develops heart disease, not every woman develops autoimmune disease, and so on), indicating that a complete understanding of vulnerability requires a finer-grained level of analysis within these subgroups.

Third, existing work has concentrated largely on the effects of stress on related biochemical measures or on a single behavioral measure (e.g., Baldwin, Wilcox, & Zheng, 1997; Acri, 1994; D'Angio, Serrano, Driscoll, & Scatton, 1988; Hofer, Wolff, Friedman, & Mason, 1972a, 1972b; Henry, Meehan, & Stevens, 1967). A literature search did not reveal any studies that have examined multiple biologic and behavioral measures within the same subjects. This omission is relevant because it is likely that the manifestation of stress vulnerability depends to some extent on the level at which stress effects are assessed. In addition, the specific level at which stress is manifested (e.g., biochemically vs. behaviorally) may provide evidence about different types of stress vulnerability that may be relevant to the prevention and treatment of stress-related disorders in humans. Further, the measurement of multiple biochemical and behavioral indices within the same subjects allows the use of statistical techniques (e.g., factor analysis, causal modeling) to construct predictive models.

The purpose of the present experiment was to address these omissions. Specifically, this experiment examined responses to mild, repeated stress in two outbred strains of rats (Sprague-Dawley and Long-Evans) within which extensive genetic variability exists but that are not bred specifically for stress responsivity. These strains were conceptualized as representing the relatively unexamined 95% of the stress vulnerability population. The experiment also evaluated responses of females as well as males to more fully determine the extent to which male vs. female stress-induced disease risk is based on the pure biologic fact of sex (as opposed to psychosocial and cultural factors that might produce gender-specific patterns of illness). Further, the experiment assessed effects of stress on multiple behaviors and biochemical indices within the same subjects in order to construct a more complete and detailed model of markers that might reveal stress vulnerability. The behavioral responses evaluated were basic unconditioned behaviors (i.e., feeding, body weight, locomotion) and behaviors that index cognitive processes, including non-volitional sensory-gating (i.e., the acoustic startle reflex with and without pre-pulse inhibition), simple working memory (i.e., passive avoidance), and search strategy efficiency and complex spatial memory (i.e., Morris water maze). The biochemical indices evaluated were the hormones of the hypothalamic-pituitary-adrenal (HPA) cortices axis [corticotropin releasing factor (CRF), adrenocorticotropin hormone (ACTH), and corticosterone].

The specific aims of this doctoral research were to: characterize stress responses in a rat model across a range of dependent variables within the same

subject; determine the extent to which stress responses depend on the sex and strain of subject; and determine the extent to which differential patterns of stress responses exist among these subgroups. A further goal of the experiment was to characterize patterns of stress responses in terms of markers of stress vulnerability vs. resilience. Markers that might indicate stress vulnerability were operationally defined as: over- or under-eating; marked gains or losses in body weight; decreases in activity and increases in anxiety indices (as revealed by different locomotion parameters); impairments in sensory-gating (as revealed by changes in startle with and without a pre-pulse); impairments in simple memory performance (indicated by passive avoidance performance); impairments in complex memory performance (indicated by Morris water maze performance); and inappropriate HPA axis hormonal responses (either hypo-reactivity or hyper-reactivity). The degree of potential stress vulnerability was inferred by determining which variables were altered by stress for each subgroup and to what degree these indices revealed maladaptive responses across and within specific subgroups. Stress resilience was defined as the absence of maladaptive changes or as the presence of improvement in behaviors as a result of stress.

The remainder of the Introduction reviews background material relevant to the present research. **Section I** presents a brief history of the stress concept, with emphasis on the part that individual differences have played in the development of the concept, and the rationale for the multilevel biobehavioral approach in this experiment. **Section II** reviews the literature on the contribution

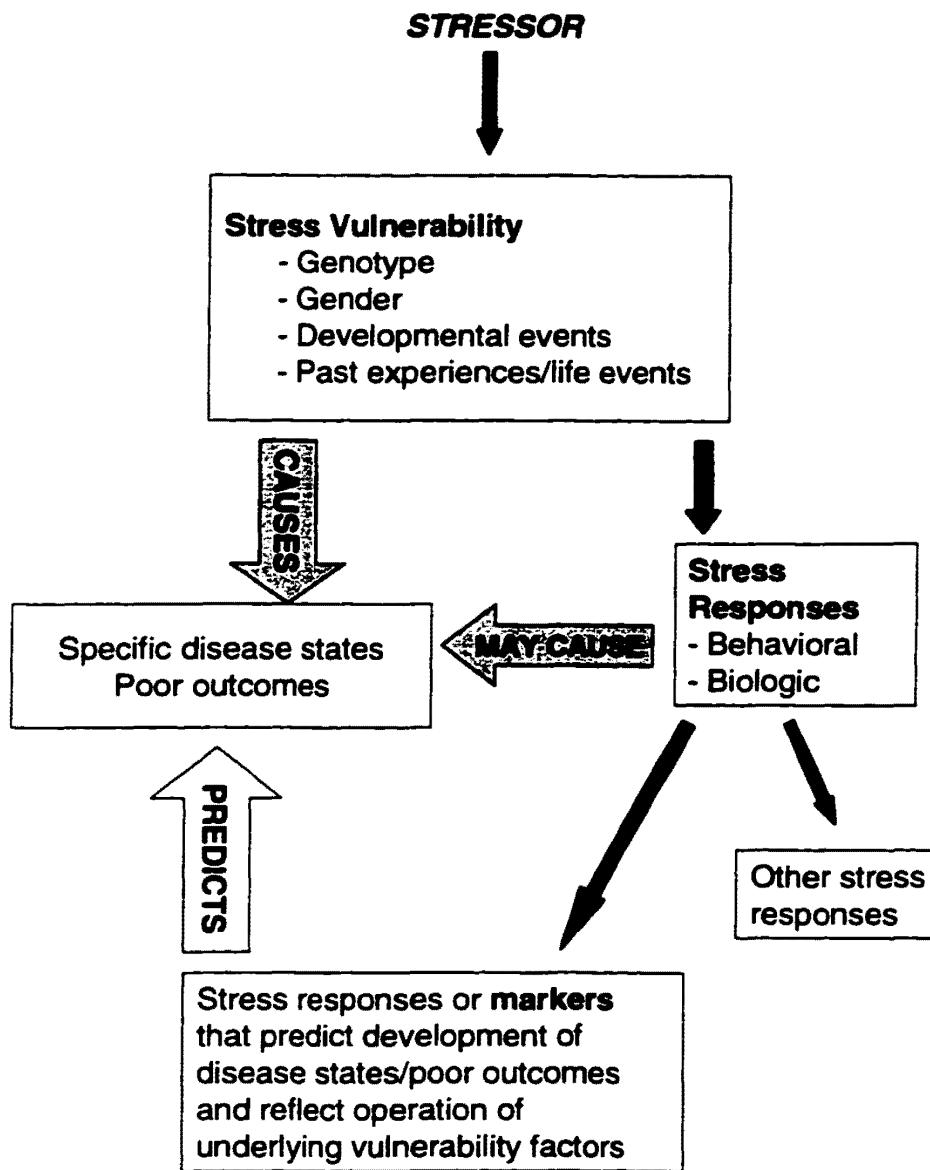
of stress to physical and psychological illness and drug use and abuse, the existence of gender differences in these consequences, and the possible contribution of other biologically-based individual differences. **Section III** presents the rationales for each independent (i.e., sex, strain, stress) and dependent variable as well as relevant past work and pilot data.

*Though the world outside us may be distressingly cold, though the heat and acid which arise from our own strenuous exertions may tend to become an overwhelming menace, we are not greatly disturbed, for our living parts touch only the body fluids which are maintained in an even and steady state. So long as this personal, individual sack of salty water, in which each one of us lives and moves and has his being, is protected from change, we are freed from serious peril (Walter B. Cannon, 1935, p. 2).*

*The fact that ...the same stressor, can cause different lesions in different individuals has been traced to what I have called “conditioning factors” that can selectively enhance or inhibit one or the other stress effect. Thus, conditioning may be internal (for example, genetic predisposition, age, or sex) or external (treatment with certain hormones, drugs, environmental elements, or dietary constituents). Under the influence of such conditioning factors (which determine sensitivity or disease-proneness), a normally well-tolerated degree of stress can become pathogenic and cause diseases of adaptation, selectively affecting predisposed body areas (Hans Selye, 1975, p. 40).*

## **I. Stress: An Organism X Environment Interaction**

The two quotes above capture the qualities that an organism brings to its potentially stressful interactions with the environment. Cannon's description of the power of homeostatic systems to buffer internal or external influences highlights the innate resilience of complex biologic systems. Selye's characterization of individual differences in stress vulnerability — “conditioning factors” — emphasizes the fact that individuals vary inherently in their resilience, in part because of biologically-based factors, such as gender and genotype. The present experiment is an animal model of individual differences in this continuum of resilience vs. vulnerability in which male and female rats of two different strains (Sprague-Dawley and Long-Evans) were exposed repeatedly to a mild stressor and multiple behaviors and biologic indices were measured.



**Figure 1.** Model of the relationships among stress vulnerability, stress-related disease states/poor outcomes, and stress responses.

The construct of stress vulnerability, the causal relationship of vulnerability to specific disease states or problematic stress-related outcomes, and the possible utility of certain behavioral and biologic stress responses that might correlate with specific vulnerability subtypes and therefore serve as markers that

predict the eventual development of specific disease states or poor outcomes are depicted in Figure 1. More specifically, the present experiment was conducted based on a series of assumptions derived from a broad reading of the stress literature. First, stress vulnerability is conceptualized as a psychobiologic attribute of the individual. Vulnerability is composed of: 1) biologically-based components, such as genotype, gender, and other factors (e.g., personality) that also appear to be biologically-based; 2) the individual's environmental history, including the presence of traumatic events during development, the accumulation of stressful life events, and associated learning and other psychological processes; and 3) the interaction of biologically-based factors with environmental history. The individual is confronted by stressors and, over time, may develop specific disease states or other problematic outcomes as a consequence of a specific vulnerability (i.e., sympathetic nervous system hyper-responsivity, neurochemical abnormalities, overactive immune system). Vulnerability, therefore, is causally linked to disease or poor outcome development, and is manifested during a time-frame of months, years, or decades.

In the short-term, the individual is confronted by stressors that result in behavioral and biological stress responses. These responses may impair health (e.g., if an individual's response to a stressful situation is to self-administer a tobacco product, then this behavioral response to stress is likely to directly result in disease) or may be correlated with the individual's specific underlying vulnerability, and therefore may have utility as predictors for the eventual

development of specific disease states. For example, hostile interview responses (i.e., in a measure designed to detect Type A personality) are correlated with a hyper-responsive sympathetic nervous system — a specific vulnerability — and are predictive of the eventual development of stress-related cardiovascular disease.

Two related aspects of responses may be useful to determine whether or not they constitute possible stress vulnerability markers: reactivity and functionality. The intensity of responses — the concept of reactivity — may be relevant because the magnitude of behavioral or biologic responses to a mild stressor might correlate with the magnitude of underlying destructive, hyper-active processes or blunted inhibitory processes that constitute a specific vulnerability. In the present experiment, the terms "reactivity" and "sensitivity" are used interchangeably.

In addition, the extent to which responses are functional and allow the organism to deal effectively with the stressor also may be relevant in identifying stress vulnerability markers. Functionality overlaps to some extent with reactivity in that extreme responses to mild stressors might not allow the organism to cope optimally. Functionality is a more complex construct than reactivity, however. For example, hypo-responsivity might indicate the absence of reactivity but also might constitute a dysfunctional response to a stressor, such as the failure to mount an immune response to an invading pathogen. In addition, the assessment of functionality depends on properties of the environment or situation in which the stressor occurs. A tripling of heart rate may be a functional

response when fleeing a gang of hoodlums but a dysfunctional response when waiting for a colleague who is late.

The goal of the experiment was to identify patterns of stress responses, including the magnitude of responses (i.e., reactivity), across dependent variables and over time that might correlate with specific human stress vulnerabilities and constitute predictors for specific stress-related diseases or negative outcomes. Although functionality was not specifically assessed in the experiment, these patterns can be conceived of as differentially adaptive. For example, an animal that loses substantial body weight, becomes hypoactive, performs poorly on cognitive tasks, and exhibits a blunted corticosterone response as a consequence of repeated exposure to a mild stressor exhibits a maladaptive pattern when compared to an animal that loses minimal weight, increases activity, improves cognitive performance, and exhibits a normal corticosterone increase. If behavioral and peripheral biochemical correlates of adaptive vs. maladaptive reactions to stress are identified in rats (the purpose of the present experiment), then the underlying mechanisms that constitute specific vulnerabilities also can be determined (the goal of future experiments).

Because behaviors in particular can be measured in humans that are identical to or analogous with those measured in rats, these correlates also may provide a model for noninvasively identifying individual humans with specific stress vulnerabilities and predicting susceptibility to specific disease states. Ultimately, this type of model also might indicate the best strategies for managing stress and preventing illness in vulnerable individuals. It is important

to note, however, that the present experiment did not investigate mechanisms that constitute stress vulnerability; this experiment focused on potential markers that might be correlated with specific vulnerabilities.

Stress is commonly defined as a process in which internal or external events — stressors — threaten or challenge an organism's existence and well-being, and stress responses occur that are directed toward reducing the event's impact (Baum, Singer, & Baum, 1981; Baum, Grunberg, & Singer, 1982; Baum et al., 1997). This definition has utility as a general conceptual model of stress but does not capture the dynamic, multilevel, and, in particular, the bidirectional quality of this organism X environment interaction in which the biology and psychology of the organism are ultimately inseparable. In addition, the relevance of individual differences to this process is not clear from this description. Both concepts — that the stress process unfolds at multiple psychobiologic levels and that important individual differences direct the process — are implicit in the history of stress and require a modern integration and extension of several different perspectives.

**Historical perspectives.** Stress as an interaction between an organism and the environment has been a unifying theme throughout the development of the concept of stress. Although historically the biological (i.e., Cannon, 1914, 1928, 1929, 1932, 1933, 1935; Selye, 1936, 1946, 1956) and psychological (i.e., Folkman & Lazarus, 1980, 1985, 1986; Lazarus & Folkman, 1984; Glass & Singer, 1972) stress traditions have conceptualized the organism and its responses in different predominant domains — a primarily biologic entity

involuntarily emitting biologic responses vs. a primarily psychological being coping psychologically and cognitively — the stress process is viewed as a dynamic interchange between the organism's resources and the environment's demands. This theme also is evident in the psychobiologic tradition (i.e., Bourne, Rose, & Mason, 1967, 1968; Mason, 1968a-e, 1975a-c; Hofer et al., 1972a, 1972b) in which the stress experience depends on psychological as well as biological factors. In all cases, stress-induced physical and mental illness is viewed as the consequence of inadequate organism resources, overwhelming environmental demands, or both.

Throughout the development of the stress concept, the possible role of biologically-based individual differences has been commented upon but has not been adequately evaluated by any of the traditions. Exemplars from these three traditions are reviewed briefly below with emphasis on how individual differences have been conceptualized and the relevance of these differences to health. Then, an integration and extension is proposed.

***The Biological Perspective. Walter B. Cannon.*** Physiologist Walter B. Cannon's classic work on the sympathetic nervous system (SNS)-generated fight-or-flight response focused on the homeostatic capacity of organisms to withstand changes in the internal (e.g., loss of blood, lack of blood glucose) or external (e.g., heat, cold, predator threat) environment (Cannon & de la Paz, 1911; Cannon, 1914, 1928, 1929, 1932, 1933, 1935, 1945). Cannon's investigations demonstrated that organisms possess physiologic systems designed to compensate, within limits, for constantly changing internal and

external physical demands. The complex orchestration of these responses was, he argued, evolutionary, adaptive, and generally self-preserving.

Cannon viewed stress as potentially destructive of health in two ways. First, when the interchange between the organism and a physical environmental demand — e.g., cold, hunger, a persistent predator — reached a point where the homeostatic system could no longer compensate, then homeostatic resistance was broken, the stress process became a “breaking strain,” and the organism was likely to suffer permanent injury or death (Cannon, 1935, p. 6). Second, responses that are adaptive when the organism is confronted with a physical threat may well be destructive when the threat is not one that can be countered by physical action. That is, the elicitation of the fight-or-flight cascade to an event — the increase in blood glucose, shunting of blood to the brain, heart, lungs, and large muscle groups, and so on — is only self-preserving when the threat can be either physically fought or actually fled. When the threat is primarily psychological, anticipatory responses that prepare the organism for exertion that never occurs become destructive of bodily systems (Cannon, 1933).

Importantly, Cannon recognized that individuals (human and animal) varied in their homeostatic capacities and reactivity to given stressors. In early studies of the effects of stress on digestion (Cannon, 1898), for example, he observed that male cats became restive when placed in the measuring apparatus and their stomach movements ceased but female cats appeared untroubled. He also noted that a well-weathered challenge for one person might

constitute another's "breaking strain." He proposed that tests could be developed to quantify the limits of individuals' homeostatic systems, to determine how these limits changed over the course of development, during various disorders, and in response to important life events and unhealthy habits (Cannon, 1935). Understanding how individuals varied in their homeostatic capacities, he proposed, would provide insight into a variety of human diseases. Despite these proposals and the observation of individual differences in the responses of experimental animals, however, **Cannon did not explicitly examine individual differences in his own work.**

**Hans Selye.** Endocrinologist Hans Selye also viewed the stress process as an interaction between organismal capacities and environmental demands that could potentially lead to disease states, but his theory of stress was conceptually distinct from the fight-or-flight response and specifically excluded homeostatic adjustments as stress responses (Selye 1936, 1946, 1956). For Selye the stress process was mediated by multiple endocrinologic systems, with emphasis on the role of the hypothalamo-pituitary-adrenocortical (HPA) axis and the multipotent bioactive products of its activation (i.e., the glucocorticoids). According to Selye, stress is the nonspecific response of the body to demands for adaptation — a deceptively simple concept that has often been misinterpreted to mean that *all* responses to adaptive demands are necessarily and by definition nonspecific. According to Selye, if a given demand for adaptation results only in specific effects — the release of insulin into the blood

in response to feeding, for example — then that demand is not a stressor and the organism is not experiencing stress. The nonspecificity that defines stress responses for Selye is a consequence of the fact that stressors by definition activate the HPA axis. The product of this activation — rising levels of glucocorticoids — are released into circulation and act throughout the body on a wide variety of tissues.

More specifically, for Selye stress-induced disease occurred when this nonspecific syndrome cycled through the three stages of the General Adaptation Syndrome (GAS): alarm, resistance, and exhaustion (Selye, 1936, 1946, 1956, 1973). Initial exposure to the stressor — the Alarm stage — resulted in production of corticotropin-releasing factor (CRF) which signaled the pituitary to manufacture adrenocorticotropin hormone (ACTH) which, in turn, stimulated the adrenal cortices to produce the glucocorticoids. If the stressor overwhelmed the body during this stage, then the organism died. If the stressor did not overwhelm the body during this initial stage, then a period of adaptation to the presence of the stressor ensued — the Resistance stage. In the Resistance stage, production of corticoids dropped to only slightly above normal as the organism adapted to the stressor. Selye characterized the Resistance stage as one of “acquired adaptation.” If the stressor continued long enough, however, for unknown reasons the organism’s capacity for adaptation was overwhelmed and the stage of Exhaustion ensued in which corticoid production again rose and multiple “diseases of adaptation,” related to the destructive effects of continued excess corticoid production, might occur.

Selye theorized about the existence of individual differences in this process, in particular that the nonspecific syndrome in response to a specific stressor also had specific, non-stress effects that depended, in part, on characteristics of the individual. These characteristics of the individual — genetic predispositions, age, gender, past experience, drug treatments, dietary factors, climate — potentially influenced individual responses to particular stressors and mediated stress vulnerability — a concept that Selye termed “energy of adaptation” (Selye, 1975). The greater the energy of adaptation conferred upon an individual by genotype and history, the greater that individual’s capacity to withstand both specific, non-stress costs of adaptation as well as nonspecific stress effects. Like Cannon, however, **although Selye theorized about the existence of individual differences, he did not empirically examine their existence or demonstrate a causal connection between particular differences, differential GAS progression, and disease vulnerability.**

***The Psychological Perspective. Richard S. Lazarus.*** Whereas the biologists Cannon and Selye (as well as many others) focused on understanding the mechanisms by which the body responded to external or internal challenges, the psychological tradition has emphasized the role of the psychological apparatus interposed between the external world and the physiology of responses and through which that world’s events are sifted and experienced. Although Richard Lazarus’ early work examined effects of psychological factors

on psychological as well as biological stress responses (e.g., Lazarus & Alfert, 1964; Lazarus, Opton, Nomikos, & Rankin, 1965), he is best-known for his predominantly psychological approach to stress.

For Lazarus, stress is an organism-environment interaction that is subjective, psychological, and cognitive. More specifically, stress is a particular relationship between the individual and the environment that is appraised by the individual as taxing or exceeding his or her resources and endangering his or her well-being (Lazarus & Folkman, 1984). For Lazarus this subjective psychological experience is a function of the cognitive-psychological factors that the person brings to the transaction — past experiences, memories, biases, early childhood influences — and of the stimuli that the environment presents to the person. That is, individuals report and experiments have demonstrated that people vary in the degree to which they experience stress in a particular situation, and the objectively-defined properties of the environmental stimulus *per se* are insufficient to explain the stress process (e.g., Folkman, Lazarus, Gruen, & DeLongis, 1986; Folkman & Lazarus, 1986, 1985, 1980; Monat, Averill, & Lazarus, 1972).

According to Lazarus, two processes mediate the person-environment relationship from within the person: cognitive appraisal and coping. Appraisal is a process of evaluation in which it is determined to what extent a particular transaction or series of transactions between the person and the environment are stressful. Coping is the process through which the person-environment relationship demands and the emotions that they generate are managed

(Lazarus & Folkman, 1984).

Coping responses can be divided into two categories: responses aimed at the problem itself — problem-focused coping — and responses aimed at managing emotional responses to the problem — emotion-focused coping (Folkman & Lazarus, 1980). Problem-focused coping includes strategies such as defining the problem, looking for alternative solutions, weighing the costs and benefits of various alternatives, choosing among the solutions, and acting on the decision. Emotion-focused coping includes cognitive processes such as distancing, minimization, avoidance, and selective attention. These strategies may lead to reappraisal of the situation as less harmful than originally believed even though the situation remains objectively the same. Emotion-focused coping also consists of behaviors that may lead to reappraisals such as seeking social support, venting anger or fear, or self-administration of alcohol or other drugs. Both types of responses potentially enhance or impair health. Deciding that the best solution for a problem is to ignore it (problem-focused coping) may enhance health when the initial threat evaluation was inaccurate or when the situation cannot be changed, but may negatively affect health if reappraisal results in the individual failing to seek medical attention for a potentially serious health problem. Similarly, emotion-focused coping strategies such as seeking social support may buffer the individual from negative stress effects (e.g., Cohen & Wills, 1985) but emotion-focused coping strategies of overeating, alcohol consumption, or other drug use may impair health.

Despite the extensive work of Lazarus and his followers on psychological

factors that produce individual differences in stress vulnerability, however, little consideration has been given to the possibility that individuals also bring certain biological strengths and weaknesses to the organism X environment interaction, and that these biological factors might influence psychological processes (i.e., appraisal) and resulting responses (i.e., choice of coping strategy). This omission may be of critical importance. For example, animal work has revealed that individual rats that exhibit the greatest behavioral and biologic responses to mild stressors also are the animals most likely to self-administer addictive drugs (e.g., Piazza, Deminiere, Le Moal, & Simon, 1989; Piazza et al., 1990, 1991). If similar links between behavioral and biologic reactivity and propensity to use addictive drugs exist in humans, then individual differences that have been conceived of as produced solely by psychological factors (i.e., choice of coping strategy) may be in fact influenced by biologic factors.

**David Glass and Jerome E. Singer.** Manipulating factors in the environment that have psychological relevance, such as the predictability and controllability of stressors, also affects the stress process. Glass and Singer (1972) found that individuals rapidly adapt to laboratory stressors such as electric shock and loud predictable (presented at regular intervals) or unpredictable noise as well as to loud noise over which they have perceived control as indexed by galvanic skin response (GSR), vasoconstriction, and electromyographic responses. The psychological variables of predictability and

controllability, however, had marked effects on complex task performance during noise and post-noise periods. Subjects who experienced unpredictable noise had more errors on the task than did subjects who experienced predictable noise, and subjects who experienced uncontrollable noise exhibited deteriorating performance over time when compared with subjects who experienced noise over which they had perceived control (i.e., a button that could be pushed to terminate the noise). In addition, subjects exposed to unavoidable shock performed more poorly after the stressor ceased than subjects exposed to shock they believed was avoidable. Further, subjects exposed to bureaucratic harassment or discrimination also exhibited post-stressor performance deteriorations.

Importantly, these studies determined that manipulation of certain key components of the individual-environment relationship — especially actual or perceived predictability and controllability of the environment — resulted in amelioration of detrimental stress aftereffects on cognitive performance. Others have demonstrated that increasing environmental demands (i.e., by making experimental tasks multiple and/or more difficult) also results in detrimental cognitive aftereffects (for review, see Cohen, 1980). Although the present experiment does not explicitly manipulate predictability or controllability over stress, others have demonstrated that animals also are sensitive to these psychological aspects of stress (e.g., Andersen, Leu, & Kant, 1987, 1988; Kant, Leu, Andersen, & Mougey, 1987; Kant, Bauman, Anderson, & Mougey, 1992) and exhibit individual differences in biologic and behavioral responses (e.g.,

Mason, 1968a-e; Klein, Popke, & Grunberg, 1997).

***The Psychobiological Perspective.*** **John W. Mason.** The organism-environment interaction theme also is common to the psychobiologic tradition exemplified by John W. Mason's work. According to Mason, the individual's experience of stress and manifestation of stress responses depends on appraisal of a situation or stimulus, personality factors and psychological defenses, situation or environmental influences, and an integrated multi-hormonal response. From Cannon, Mason took the idea that endocrinologic responses to stress are evolutionarily functional reactions associated with anticipated need for exertion, are homeostatic, and are aimed at preserving the organism. His work demonstrated, however, that the patterns of these responses exhibited marked individual differences in humans as well as in animals (e.g., Bourne, Rose, & Mason, 1967, 1968; Mason, 1968a-e; Poe, Rose, & Mason, 1970; Hofer et al., 1972a, 1972b), indicating that the stress experience was not adequately defined by objective descriptions of the stressor. In fact, these differences in biological indices were mediated by psychological factors such as the presence of predictability or control in the environment, specific cognitive defenses employed, the role of the individual within the group, and the individual's personal history.

In particular, Mason proposes that these multihormonal responses reflect:

- 1) intrapsychic adaptations to stressors, including unconscious or repressed processes;
- 2) cognitive processes of appraisal;
- 3) the balance between various types of coping processes;
- 4) trait (stable) as well as state (transitory)

adaptations to stressors (Mason, 1970, 1974, 1975; J.W. Mason, personal communication, 1/25/99). The relationships between hormones and the brain also are conceived of as bidirectional, with hormones both reflecting and modulating intrapsychic processes (J.W. Mason, personal communication, 1/25/99).

**The link between stress and disease is this highly individual psychosomatic interplay (Mason, 1970, 1974, 1975; J.W. Mason, personal communication, 9/14/98). Further, Mason argues that the measurement of multiple intertwined and interacting neuroendocrine responses provides a window into this process, and ultimately a predictive tool regarding the likelihood of mental or physical disease and prognosis for recovery.**

**Although Mason and colleagues have focused on characterizing the existence of individual differences in stress responses, this model of stress vulnerability is limited to neuroendocrine indices. The possible relevance of behavior, therefore, is not considered but is an important level of analysis because it may reflect integrated neuroendocrine functioning. Given the complexity of neuroendocrine patterns, a model that includes characterizations of behavioral patterns in an attempt to predict maladaptive responses to stress (e.g., the present experiment) may provide information that is a valuable complement to neuroendocrine indicators or that can replace these indices partially or entirely.**

**An Integration and Extension. Based on the available literature on**

stress, it seems that a complete theory of stress would integrate subjective psychological existence with the fact that the psychological world is grafted to and inseparable from biologic processes. These two components could be conceived of as different kinds of forces that operate during the stress experience: psychological forces that are the perceptible core of human existence — awareness of self, the relevance of the threat or challenge to self, and cognitive and behavioral responses aimed at preserving self; and biologic forces that by themselves lack consciousness and operate largely beneath the level of psychological awareness, that are evolutionarily old, and that follow their own mute teleology of homeostasis — another form of self-preservation.

This model, however, in which biologic and psychological forces are orthogonal, would be inadequate. In actuality the forces are interdependent and operate in a complex bidirectional manner. Therefore, a unitary model of stress proposes that the “nonbiological” stress responses — emotional, cognitive, psychological — and the “biological” stress responses are conceptually inseparable and constitute a mind-body holism. By extension, the stress process cannot adequately be characterized with psychological reductionism (i.e., the exclusive measurement of psychological and cognitive responses) or with biological reductionism (i.e., the exclusive measurement of biologic responses).

This integration builds on the substantial work of others (i.e., Cannon, Selye, Lazarus, Glass, Singer, Mason) and is central to the biobehavioral approach utilized in the present experiment. Several methodologic consequences flow from utilizing this conceptualization of stress. First, it is

necessary to measure multiple responses (i.e., in a rat model, behaviors as well as biologic indices) that triangulate on the same phenomenon as well as multiple levels of response (e.g., Baum et al., 1982; Grunberg & Singer, 1990; Baum & Grunberg, 1995). In the present experiment, for example, behavioral measurements of cognitive performance in rats ranged from simple sensory-gating of a stimulus (pre-pulse inhibition of the acoustic startle reflex) to a simple memory task (shuttlebox passive avoidance) to a complex learning and memory task (performance in the Morris water maze). Similarly, multiple stress hormones were measured (e.g., CRF, ACTH, corticosterone).

Because the effects of stress unfold over time in an organism X environment interaction, it also is critical to measure responses more than once to capture the process nature of this dynamic interchange. Therefore, in the present experiment, where possible, behavioral responses were measured at multiple time points. Indications of the stress vulnerability of rat subgroups (i.e., males vs. females, Sprague-Dawleys vs. Long-Evans) may emerge over time. This approach also makes clear the importance of measuring behaviors that allow inferences about psychological and cognitive processes (e.g., cognitive tasks, open field behaviors) as well as biologic responses (e.g., peripheral hormones).

## **II. Individual Differences in the Relationship Between Stress and Disease**

The stress process is believed to negatively affect health in three ways: 1) by direct actions on the biologic systems of the body and brain; 2) by indirect effects on health-relevant behaviors that may be a consequence of direct stress effects; and 3) by indirect effects on behaviors that influence treatment of illness (Krantz, Glass, Contrada, & Miller, 1981). Examples of direct stress effects include chronically elevated blood pressure that leads to increased likelihood of stroke or degeneration of hippocampal cells from exposure to chronically elevated glucocorticoids (Baum et al., 1997; McEwen, De Kloet, & Rostene, 1986; Lupien et al., 1997). Stress can indirectly affect health by increasing the likelihood of engagement in health-impairing behaviors such as overeating, cigarette-smoking, alcohol consumption, and other substance abuse (Cohen, Evans, Stokols, & Krantz, 1986). These behaviors may occur in an effort to manage unpleasant stress symptoms, such as feelings of anxiety or depression, to distract, or because restraints are lifted. Stress can affect behaviors that influence treatment of illness when individuals fail to report symptoms and seek medical care or do not comply with medical regimens (Baum et al., 1997).

The fact that some but not all individuals exposed to a given stressor respond in maladaptive ways through the development of physical or mental illness or engagement in health-harming behaviors is well-known (e.g., McEwen, 1998). As understanding of the multicausal nature of physical and psychological disease states has deepened, it has become clear that the stress process is a complex, psychobiologic individual X environment interaction.

Several lines of evidence suggest that differences in stress vulnerability are biologically-based to some extent and can be considered to be the result of genotype broadly defined. Genotype can be conceived of as a biological, "hard-wired" individual difference. In the broadest sense, genotype is manifested by gender and ethnicity in humans and sex and strain in rats. Organisms of different genotypes may have different thresholds for activation and de-activation of stress-sensitive responses, different rates of stress hormone synthesis and metabolism, and/or different densities, distributions, or sensitivities of relevant peripheral and central receptors. The operation of genotype in responses to stress can be inferred at several levels.

**The relevance of gender.** One obvious manifestation of the relevance of genotype to differential stress vulnerability is revealed by gender — the biologic status of an individual as male or female. There are gender differences in a variety of disease risks, including cardiovascular disease, autoimmune diseases, psychological disorders, and the likelihood of engaging in particular health-imparing behaviors such as substance abuse. These gender differences are important because the directionality of the differences may provide clues about causal mechanisms.

With regard to cardiovascular disease, stress is a risk factor for atherosclerosis, hypertension, stroke, myocardial infarction, and sudden cardiac death (Krantz et al., 1996), but men are more likely than are women to die of cardiovascular diseases throughout most of life, in part because of the protective effects of estrogen (Lerner & Kannel, 1986). Stress also can impair immune

function (Glaser, Kiecolt-Glaser, Speicher, & Holliday, 1985; Kiecolt-Glaser & Glaser, 1987; Cohen & Williamson, 1991) but women are more likely than are men to develop autoimmune diseases and to die of pneumonia or influenza (Verbrugge, 1985). Further, stress has been implicated as a factor in the development of psychological illness (Anisman & Zacharko, 1992; Baum, Cohen, & Hall, 1993). Again, there are large gender differences in disorder prevalence. The majority of panic disorder patients are female (estimates range from 60-90%) as are the majority of phobia sufferers (75-90%) (Rapee & Barlow, 1993). Women also are up to three times more likely to be depressed than are men (Rehm & Tyndall, 1993). The likelihood of engaging in particular health-impairing behaviors also varies by gender. Men and women are equally likely to smoke (SAMHSA, 1996a, 1996b) but men are more likely than are women to drink alcohol and use illicit drugs (Lex, 1991). Women are more likely than are men, however, to be obese, bulimic, or anorectic (Andersen, 1990; Bellack & Hersen, 1993). **These epidemiologic sex differences in stress-related disorder prevalence are consistent with the idea that males vs. females have different behavioral and biologic responses to stress.**

These epidemiologic patterns also have been substantiated in the laboratory and in animal studies. In the laboratory, men exhibit greater increases in blood pressure and higher levels of catecholamines than do women during or immediately after an acute stressor, such as a laboratory challenge, but women exhibit larger heart rate increases than do men (e.g., Dembroski, MacDougall,

Cardozo, Ireland, & Krug-Fite, 1985; Frankenhaeuser, Dunne, & Lundberg, 1976; Stoney, Davis, & Matthews, 1987; Stoney, Matthews, McDonald, & Johnson, 1988; Frankenhaeuser, 1979). These differences in responses to laboratory stressors may be relevant to the different patterns of cardiovascular disease observed in men vs. women because increases in blood pressure and catecholamine release (exhibited by men) are associated with arterial damage and plaque formation (Baum et al., 1997).

Men and women also exhibit differential activation of the HPA axis in response to corticotropin releasing factor (CRF). Specifically, women exhibited greater adrenocorticotropin (ACTH) responses and more prolonged cortisol elevations than did men (Galluci et al., 1993). In light of the immune-suppressing actions of cortisol, the increased reactivity of women's HPA axis may be relevant to female's greater rates of immune dysfunction and death from infectious disease.

Feeding patterns of men vs. women also differ in response to stressors in the laboratory. Men decrease or do not alter eating in response to stress but women increase consumption of high-carbohydrate, fat-containing foods (Grunberg & Straub, 1992; Grunberg & Klein, 1995; Klein, Faraday, & Grunberg, 1996). This phenomenon may be related to the greater prevalence of eating disorders among women. Together, the human epidemiologic and experimental data indicate that the genders respond differently to stress across a range of behaviors and biologic indices and that the specifics of these differences may be causal in the stress-disease relationship.

**The relevance of other biologically-based differences.** Other individual differences exist, however, in patterns of stress-related physical and mental illness and changes in appetitive behaviors that imply the possible operation of biologically-based factors other than or in addition to gender. The simplest manifestation of these other differences is the fact that, despite the prevalence of stress in modern life, all men and women do not develop stress-related disorders. In addition, individuals exposed to serious, life-threatening stressors do not all develop stress-related disorders. For example, the percentage of individuals who develop post-traumatic stress disorder (PTSD) in response to disasters, such as devastating tornados, cyclones, or volcanos, or serious motor vehicle accidents ranges from 2% to 18% (Fairley, 1984; Steinglass & Gerrity, 1989; North, Smith, McCool, & Lightcap, 1989; Delahanty et al., 1996).

Individual variability in reactivity to stressors may account for some of these differences. Differential patterns of stress reactivity have been identified across a range of biological responses. For example, the Type A behavior pattern of excessive competitive drive, impatience, and hostility first described by Friedman and Rosenman (1959) as a risk factor for coronary heart disease has been characterized by Krantz and Durel (1983) as one in which individuals at risk display excessive sympathetic responses to events, and it is these biologic responses that drive Type A cognitions and behaviors. With regard to corticosteroid responses, several laboratory studies have demonstrated that individual differences exist in cortisol reactivity to stressors such as exercise or

public speaking, with some individuals exhibiting large cortisol increases and others exhibiting relatively small increases (Petrides et al., 1994, 1997; Lupien et al., 1994, 1995, 1997). Among rodents, psychosocial stressors (unstable social groups) produce hypertension in normotensive strains of animals (Henry, Ely, & Stephens, 1972; Henry et al., 1967; Henry, Stephens, & Santisteban, 1975; Henry & Stephens, 1981; Henry, Stephens, & Vander, 1983; Henry et al., 1993). The extent to which disease is produced, however, depends on the strain of animal with Long-Evans rats exhibiting the greatest blood pressure increases, in part because of strain-dependent changes in catecholamine synthesis (Henry et al., 1993).

To some degree, differences in stress effects are likely the result of culture, environment, social support, and cognitive variables such as the decision to refrain from overeating, to exercise, or to restrict use of certain substances. Animal studies — in which environmental variables can be controlled and factors of culture and conscious cognitive processes have been excluded — indicate, however, that biologic and behavioral reactivity to stress, disease risk, propensity for substance use, and quality of cognitive task performance under stress are linked by the common consequences of certain peripheral and central biochemical cascades (e.g., Broadhurst, 1960; Piazza et al., 1989, 1990, 1991; Sternberg et al., 1992; Henry et al., 1993).

The relationships between behavioral and biochemical reactivity to stress, disease, substance self-administration, and cognitive performance have been examined with specific outbred and inbred rat strains bred for relative stress

responsivity. Outbred Maudsley reactive (MR) and non-reactive rats (MNR), for example, were originally selected and inbred for high and low defecation scores in an open field as an index of autonomic reactivity (Broadhurst, 1960). The two strains have been studied extensively because they differ in fear-motivated performance and other behavioral and biochemical responses to stress. The MNR strain (low defecating) learns better under stress and exhibits fewer freezing behaviors in fear-provoking situations than does the MR strain (Savage & Eysenck, 1964; Imada, 1971). These differences appear to be the consequence of differences in central and peripheral norepinephrine concentrations and metabolism in resting as well as stress-induced states (Slater, Blizzard, & Pohorecky, 1977; Liang & Blizzard, 1978; Blizzard, 1988; Buda et al., 1994) as well as differences in serotonergic and GABA-ergic systems (Sudak & Maas, 1964; Rick, Huggins, & Kerkut, 1967). Similarly, the Roman high (RHA) and low avoidance (RLA) strains were bred for differential acquisition of a passive avoidance task and also differ in other fear-motivated behaviors (Driscoll & Bättig, 1982). These behavioral differences have been linked to differences in central dopamine metabolism (D'Angio et al., 1988). It is important to note, however, that because of inbreeding the responses of these special strains to stressors represent the ends of a continuum and model extreme stress phenotypes of hyper- and hypo-reactivity.

Among inbred strains, stress response differences are more extreme and the link between stress reactivity, addictive behaviors, and disease propensity (i.e., immune function) is most clearly seen. For example, the inbred Fischer-

344 and Lewis strains differ markedly in biochemical responses to stress. Fischer-344 rats have higher basal corticosterone levels than Lewis rats, and are hyper-responsive to stressors as indexed by corticosterone and CRF; Lewis rats are hypo-responsive by the same measures (Sternberg et al., 1989, 1992; Dhabdar, McEwen, & Spencer, 1993). The divergent responses to stress of Fischer-344 and Lewis rats have been linked to differential susceptibility to inflammatory autoimmune diseases and to different propensities to self-administer drugs of abuse (e.g., ethanol, cocaine, morphine). For example, Lewis rats are more susceptible to arthritis than are Fischer-344 rats, in part because of chronically low levels of corticosterone, a potent anti-inflammatory hormone (Griffin & Whitacre, 1991; Sternberg et al., 1989). Lewis rats also consume more cocaine, alcohol, morphine, and barbital than do Fischer-344 rats (George, 1990, 1991; Suzuki et al., 1988; Suzuki, Motegi, Otani, Koike, & Misawa, 1992; Suzuki, Koike, Yanaura, George, & Meisch, 1987). These differential vulnerabilities appear to result, in part, from a defect in corticotropin-releasing factor (CRF) biosynthesis in Lewis rats (resulting in abnormally low corticosterone levels) and different levels of tyrosine hydroxylase in dopaminergic brain reward systems between the strains (Beitner-Johnson, Guitart, & Nestler, 1991; Griffin & Whitacre, 1991; Sternberg et al., 1989, 1992). Again, it is important to note that these strains represent narrow extremes on the continuum of reactivity to stress, leaving the greater portion of the continuum unmodeled. The widespread prevalence of stress-induced disease in humans, however, indicates that more than the tails of this distribution are relevant to

understand the stress-disease relationship.

In support of this point, differential stress reactivity and some of its consequences (i.e., different responses to drugs) also have been demonstrated within outbred strains that are not bred for specific behavioral or biologic responses to stress (i.e., Sprague-Dawley, Long-Evans) and, therefore, that can be conceived of as representing the middle of the distribution. For example, male Sprague-Dawleys that exhibited the largest acoustic startle and pre-pulse inhibition PPI responses at baseline also exhibited the greatest startle and PPI increases in response to nicotine administration or to immobilization stress (Acri, 1994). Male rats (strain not reported) that exhibited the largest catecholamine responses to stress also demonstrated the largest decreases in catecholamines when given ethanol (Livezey, Balabkins, & Vogel, 1987). Sprague-Dawley males that exhibited the greatest locomotor responses to stress and to a single injection of amphetamine were the most likely to develop subsequent amphetamine self-administration (Deminiere, Piazza, Moal, & Simon, 1989; Deminiere et al., 1992). Mechanisms for these differences appear to be related to differential corticosterone reactivity and the properties of corticosterone to modify activity of the mesocorticolimbic dopaminergic system (De Kloet, 1991; Faunt & Crocker, 1988; Rothchild et al., 1984). Interestingly, rats will orally self-administer corticosterone alone (Deroche et al., 1993), consistent with the idea that corticosteroids modify dopaminergic reward pathways. Exploratory responses to novelty also are positively correlated with peripheral corticosterone levels, propensity to self-administer various drugs, responsivity to food reinforcement,

and activity of dopaminergic systems (Dellu, Mayo, Le Moal, & Simon, 1993; Piazza et al., 1989, 1990, 1991; Dellu, Piazza, Mayo, Le Moal, & Simon, 1996). Importantly, these studies have focused for the most part on the responses of male Sprague-Dawley rats. Whether similar patterns exist in responses of female Sprague-Dawleys and of Long-Evans rats is not known but may be relevant to modeling of a genotypically varied human population. In addition, most of this literature has examined the relationship between one biochemical variable and one behavioral variable. The manifestation of stress reactivity across several biologic indices and behaviors in the same animal, therefore, has not been assessed but also may be important to understand specific types of stress vulnerability.

Taken together, the human and animal literatures indicate that biologically-based individual differences may mediate reactivity to stress that are directly or indirectly relevant to physical and psychological health and illness. The animal literature in particular indicates that these differences are causally related to differences in underlying neurochemistry, and that HPA axis hormones may be a critical component of these outcomes. **There are large gaps, however, in this literature.** First, most of the work done in rats has focused on **extreme stress phenotypes** — leaving the range of responses between the two extremes relatively unexamined. This omission is important because effects of stress on health are widespread and are not limited to a small proportion of individuals.

Second, this literature has focused largely on the responses of male animals, leaving the stress responses of female rats mostly unevaluated. This gap is of critical importance because large gender differences exist in human stress-related physical and psychological illness, indicating that sex is an important variable in certain types of stress vulnerability.

Third, existing work has concentrated largely on the effects of stress on related biochemical measures or on a single behavioral measure. No studies have examined multiple biologic and behavioral measures within the same subjects. This omission renders current understanding of stress vulnerability incomplete because manifestation of stress vulnerability may depend on the level at which stress effects are assessed. In addition, the specific level at which stress is manifested (e.g., biochemically vs. behaviorally) may provide evidence about different types of stress vulnerability that may be relevant to the prevention and treatment of stress-related disorders in humans. All of these gaps can be addressed with the use of an animal model of genotypically-varied subjects of both sexes exposed or not exposed to repeated mild stress and the measurement of multiple behaviors and biochemical indices within the same subjects.

### **III. Strain, Sex, and Stress in a Biobehavioral Animal Model**

This section reviews material relevant to each independent and dependent variable of the proposed research. The experiment used male and female rats of two strains (Sprague-Dawley and Long-Evans) exposed or not exposed to mild, repeated immobilization stress. The behavioral dependent measures constitute two domains: 1) body weight, feeding, and locomotion; and 2) cognitive processes (i.e., acoustic startle with pre-pulse inhibition [PPI], passive avoidance, and Morris water maze). The goal of using this range of behavioral dependent variables within the same animals was to provide a detailed picture of stress response patterns within and across domains that might reveal differential underlying stress vulnerabilities. The biologic dependent variables were the hormones of the HPA axis (CRF, ACTH, and corticosterone). These hormonal indices were selected to validate the stressor and to determine whether behavioral differences among subgroups of stressed animals might be related to differences in HPA axis activity. Relevant behavioral and biologic pilot data also are presented.

**Independent Variables. Strain.** The present experiment used adult male and female rats of the outbred Sprague-Dawley and Long-Evans strains. These strains were selected based on serendipitous findings in the context of another experiment. Specifically, the data presented below as pilot work were the no stress and stress cells of a larger, full factorial experiment that examined behavioral responses to nicotine, to stress, and to nicotine in combination with

stress. That experiment was undertaken to replicate and extend findings regarding strain differences between Sprague-Dawley and Long-Evans rats in behavioral responses to nicotine (i.e., Faraday et al., 1998; Faraday, O'Donoghue, & Grunberg, 1999). The results from that experiment revealed that the two strains also exhibited differential behavioral responses to immobilization stress — a new finding. This new finding was the empirical basis for using these strains in the present experiment as a model of stress vulnerability.

In addition, these strains are widely used for a variety of experimental questions, including studies concerned with stress, immune function, patterns of drug addiction, and the aging process, and are not bred specifically for particular behavioral or biologic responses. As with different human phenotypes, the two strains are genetically more alike than they are different but can vary at each allele (Pat Mirley, Charles River Laboratories, personal communication, 11/19/97). The two strains differ phenotypically in one obvious way: Sprague-Dawleys are albinos with white coats and unpigmented retinas and Long-Evans are pigmented rats with black or brown and white coats and pigmented retinas.

Even though inbred strains exist that are bred specifically for stress hypo- and hyperresponsivity (e.g., Fischer-344 and Lewis), these strains were not proposed for use in the present experiment. Use of inbred strains that respond differently to stress (and with little within-strain variability because of the shared, histocompatible genotype produced by at least 20 generations of brother-sister matings, analogous to monozygotic twins) (Crabbe & Phillips, 1990) is useful to understand the mechanisms that account for extreme stress phenotypes.

However, the fact that these strains represent narrow extremes of stress responses also indicates that they represent the tails of an underlying and theoretically normal distribution of responses.

When the experimental goal is to model a broadly variable human population, the use of outbred strains not specifically bred for stress reactivity is preferable. In the maintenance of these strains, brother-sister matings are explicitly prevented to maintain genetic diversity. This diversity is evidenced by that fact that within outbred strains individual animals can differ at each allele and also at every major histocompatibility complex (MHC) locus (Pat Mirley, Charles River, personal communication, 11/19/97) just as in the human population.

Importantly, sufficient differences exist between Sprague-Dawleys and Long-Evans and sufficient variability exists within each strain to make the experimental goal of further characterizing these differences in terms of their relevance to stress vulnerability feasible and relevant (see sections below under Dependent Measures). In addition, the two strains differ across several neurochemical indices that may be relevant to stress reactivity, and ultimately to stress vulnerability. For example, Sprague-Dawley rats metabolize tryptophan faster than do Long-Evans rats (Costa et al., 1982), and the strains differ in tyrosine hydroxylase activity (the rate-limiting enzyme in the synthesis of the catecholamines dopamine, norepinephrine, and epinephrine), with Long-Evans rats having greater activity in the brainstem and hypothalamus than Sprague-Dawley rats, and Sprague-Dawleys having greater activity in the adrenals than

Long-Evans rats (Park, Park, Joh, Anwar, & Ruggiero, 1990).

The strains also differ in behavioral and biologic responses to some drugs. These differences may be relevant in the present experiment as indirect evidence of differential underlying mechanisms of stress actions and potential vulnerabilities. For example, nicotine administration has opposite effects on startle and PPI in the two strains, increasing startle and PPI of Sprague-Dawley rats (Acri, 1994; Acri, Brown, Saah, & Grunberg, 1995; Acri, Morse, Popke, & Grunberg, 1994; Faraday et al., 1999a) but decreasing startle and PPI of Long-Evans rats (Faraday, Rahman, Scheufele, & Grunberg, 1998; Faraday et al., 1999a). The two strains also differ in locomotion responses to some serotonergic agonists (e.g., cocaethylene, a neuroactive metabolite of concurrent cocaine and alcohol consumption), possibly as a result of differences in central serotonin bioavailability (Horowitz, Kristal, & Torres, 1997). Sprague-Dawley rats voluntarily consume more ethanol than do Long-Evans rats (Gauvin, Moore, & Holloway, 1993) and Long-Evans rats exhibit greater motor impairments and greater reductions in hippocampal neuronal activity after ethanol consumption than do Sprague-Dawley rats (Sellin & Laakso, 1987). Long-Evans rats also are more sensitive to the anxiolytic behavioral effects of benzodiazepines than are Sprague-Dawley rats (Onaivi, Maguire, Tsai, Davies, & Loew, 1992). These differential drug responses suggest that the two strains bring different behavioral and biologic factors to the organism X environment stress interaction. Further, differential drug responses may be useful in determining the mechanisms by which stress differentially alters behaviors, especially because similar neural

pathways are activated by reinforcing drugs and stress.

Studies that have compared stress responses of Sprague-Dawleys and Long-Evans directly indicate that the nature of the stressor is a critical variable. Long-Evans male rats develop more stomach ulcers than do Sprague-Dawley males when exposed to lengthy periods of water-restraint stress (e.g., 2 hours) (Pare, 1989). The strains also differ in stress-induced potentiation of analgesia, with morphine's analgesic effects increased by restraint stress in Sprague-Dawley rats, but not in Long-Evans rats (Woolfolk & Holtzman, 1995). The strains also differ in hypertensive responses to chronic cold stress, with Sprague-Dawley rats developing greater systolic and diastolic blood pressure elevations, more cardiac hypertrophy, and increased urinary catecholamine output while the same indices in Long-Evans rats exhibited minimal changes (Riessmann, Baron, Fregly, & van Bergen, 1992). When the stressor is colony social instability, however, Long-Evans rats exhibited much greater blood pressure increases, greater aggression toward conspecifics, and greater weight loss (Henry et al., 1993). Strain differences in stress responses also exist with regard to the some of the behavioral and biologic measures proposed for use in the present experiment. These differences are reviewed under **Dependent Variables**.

**Sex.** The rationale for including rats of both sexes follows from the experimental goal of modeling a variable male and female human population. As with the variable of strain, pilot work indicates that there are sufficient

differences between male and female Sprague-Dawley and Long-Evans rats and sufficient variability within each sex to make the experimental goals reasonable (see **Dependent Variables**).

There is relatively little published work on sex differences in rat stress responses. It has been reported, for example, that female Long-Evans rats exhibited greater corticosterone and prolactin responses to acute stressors, greater binding at 5HT<sub>1A</sub> hippocampal receptors, greater increases in benzodiazepine receptors post-stress, and reduced rates of dopamine synthesis and turnover when compared to Long-Evans males (Mendelson & McEwen, 1991; Wilson & Biscardi, 1994; Demarest, Moore, & Riegle, 1985). The corticosterone rise in response to a variety of stressors is faster and greater in female Sprague-Dawleys than in males (Kant et al., 1983; Livezey et al., 1987; Baldwin et al., 1997). Living in an enriched environment reduces fear-related behaviors (e.g., freezing) of female Sprague-Dawleys but not male Sprague-Dawleys when confronted with a live predator (a cat) (Klein, Lambert, Durr, Schaefer, & Waring, 1994). Female Sprague-Dawleys also appear to experience less stress-induced analgesia than do male Sprague-Dawleys (Romero & Bodnar, 1986; Apatov, 1998). Pilot work also indicates that sex differences exist in both strains that are relevant to the proposed experiment (see **Dependent Variables**).

**Stress Manipulations: Immobilization.** Immobilization or restraint is a nonpainful physical stressor that is widely used in stress investigations with rats

as subjects. The stressful nature of the experience is believed to derive from the fact that rodents find forced immobility aversive, even when the restraint is nonpainful. Exposure to immobilization reliably produces elevations in stress hormones, including peripheral ACTH, beta-endorphin, prolactin, corticosterone (Kant et al., 1983, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Shaham, Alvares, Nespor, & Grunberg, 1992; Acri, 1994; Faraday & Grunberg, unpublished data). Brief exposures (e.g., 15-20 min) are sufficient to elicit some of these responses, such as elevated corticosterone and ACTH (Acri, 1994; Faraday & Grunberg, unpublished data) and constitute a mild physical stressor.<sup>1</sup> Brief exposure also is sufficient to reliably alter behaviors and other indices, including feeding, body weight, acoustic startle responses, and locomotion (see **Dependent Variables**).

**Dependent Variables.** Background material regarding each dependent variable is reviewed below. Each variable is defined and the rationale for its inclusion is discussed. Then, the relevant stress literature is presented and available pilot data are provided.

**Body weight and feeding.** Body weight and feeding behavior can be used as general indices of animal health and growth. In the present experiment these variables are included because changes in body weight and feeding occur in response to stress and may be relevant to maladaptive stress-related eating

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<sup>1</sup>

It is important to note that immobilization is a physical stressor rather than a psychological stressor. It is possible, therefore, that experimental results are relevant only to physical stress situations rather than to psychological stress — the more common stressors in the human condition.

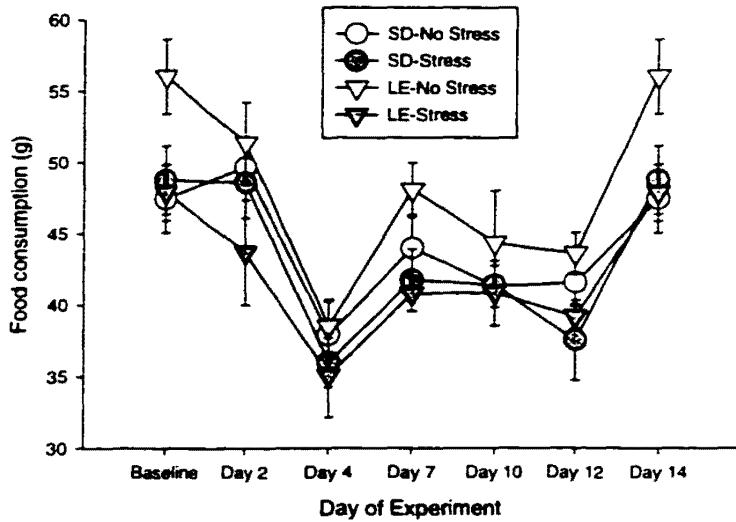
patterns (e.g., undereating or overeating) or metabolic changes. Published reports indicate that, in rats, there are sex differences in immobilization effects on body weight and feeding that depend on the duration of the stressor. For example, exposure to one or two hours of immobilization reduced feeding of both male and female Sprague-Dawleys (Krahn, Gosnell, Grace, & Levine, 1986; Donohoe, Kennett, & Curzon, 1987; Marti, Gavalda, Jolin, & Armario, 1993). When immobilization occurred for 20 min/day, however, females, but not males, habituated to these feeding effects (Zylan & Brown, 1996).

#### Pilot work using

Sprague-Dawley and Long-Evans rats revealed that feeding and body weight responses to 20 min/day immobilization depended on the strain as well as on the sex of rat (Faraday, Hygge, Rahman, & Grunberg, 1998).

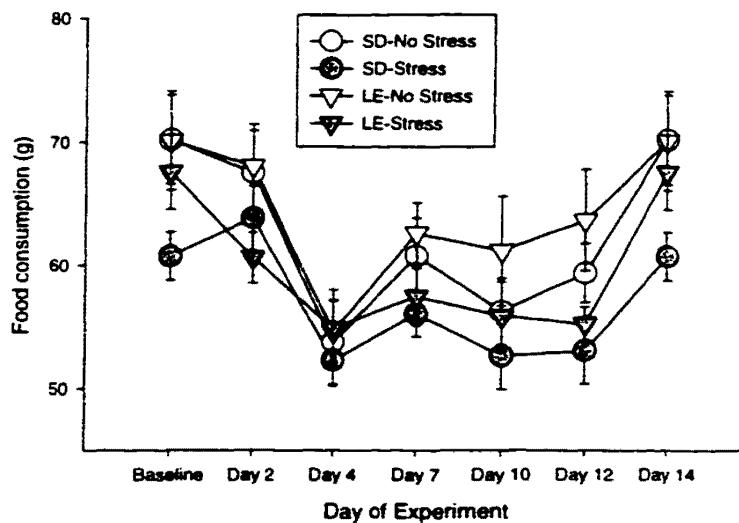
Male rats decreased feeding regardless of strain, Sprague-Dawley female feeding did not change under stress (consistent with Zylan & Brown, 1996), and Long-Evans female feeding was reduced by stress (see Figures 2 and 3).

Because body weight is the outcome of energy intake (feeding) and



**Figure 2.** Food consumption (during 2-day periods) of Sprague-Dawley and Long-Evans females.

energy expenditure (physical activity and metabolism), measures of feeding, activity (i.e., locomotion), and metabolism (i.e., corticosterone) also can be incorporated into a predictive model of body weight in order to examine possible causal inter-relationships among these variables.



**Figure 3.** Food consumption (during 2-day periods) of Sprague-Dawley and Long-Evans males.

**Locomotion.** Locomotor activity is a collection of sensitive unconditioned behaviors that occur when an animal ambulates in its environment. Aspects of rat locomotion are widely used to index effects of manipulations, including stress effects, and to pinpoint specific neuroanatomical and neurotransmitter and receptor-level mechanisms for these effects (e.g., Roberts, Lessov, & Phillips, 1995; Acosta & Rubio, 1994; Lemoine, Armando, Brun, Segura, & Barontini, 1990; Plaznik, Stefanski, Palejko, & Kostowski, 1992). Locomotion typically is measured repeatedly within the same experiment, and individual animals exhibit consistent responses over time (i.e., high-active animals maintain high activity levels and low-active animals maintain low activity) (e.g., Consroe, Boren, & Hsu, 1982; Sanberg, Moran, Kubos, & Coyle, 1983; Nichols & Schreur, 1987; Young & Johnson, 1991; Schreur & Nichols, 1986; Roberts et al., 1995; Acosta &

Rubio, 1994; Lemoine et al., 1990; Plaznik et al., 1992; Faraday & Grunberg, 1999; Faraday, Scheufele, Rahman, & Grunberg, 1999). Locomotion is included in the present experiment to index general arousal and activity, exploratory behavior, and possible anxiety or fear.

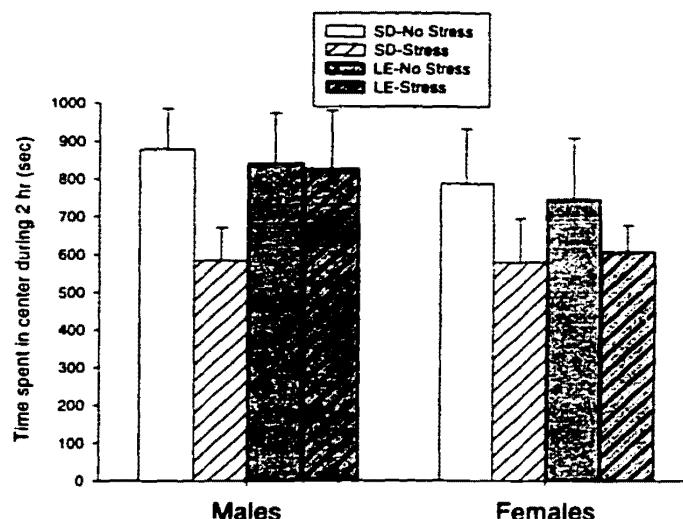
More specifically, locomotor activity includes measures of activity in the horizontal plane, distance traveled, rearing behavior or vertical activity, and time spent in the center vs. in the margin of an open field. Because rodents may experience open field situations as aversive, locomotor activity also is used as a possible index of emotional states in rats (e.g., fear, anxiety) and of manipulation effects to alter emotional states. For example, changes in locomotor behaviors are used as drug screening tools in order to determine the general arousal and anxiolytic properties of compounds (e.g., Consroe et al., 1982; Sanberg et al., 1983; Nichols & Schreur, 1987; Young & Johnson, 1991; Schreur & Nichols, 1986).

Locomotion is a useful behavior to index subjective effects of stress because different aspects of locomotion (e.g., horizontal activity and total distance, vertical activity, time spent in the margin vs. center of an open field) have been interpreted to reflect different physiological or emotional states (i.e., arousal, exploration, and fearfulness or anxiety, respectively) (e.g., Ader & Conklin, 1963; Archer, 1973; Walsh & Cummins, 1976; Nichols & Schreur, 1987; Crawley et al., 1997; Faraday et al., 1999b). Different subjective states in humans can be conceptualized in an animal model as being made up of these separate components. For example, increased anxiety as a result of stress

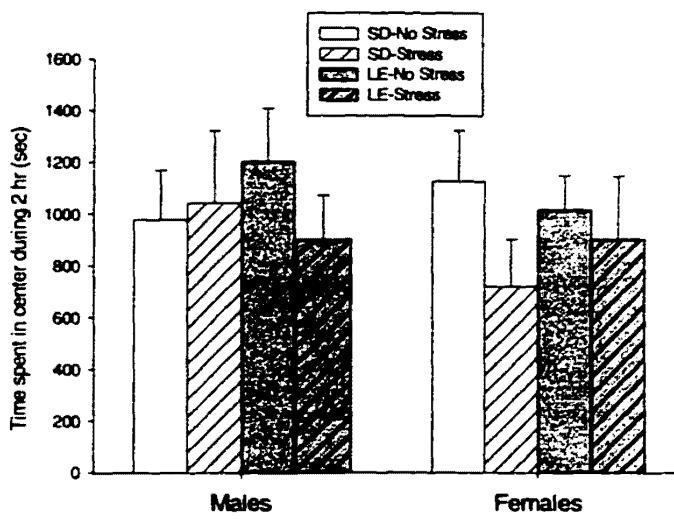
exposure might be reflected in increased time spent in the margin of the open field. An increase in total distance traveled without a change in the amount of time spent in the margin vs. in the center of the field might indicate that stress increased arousal but did not affect anxiety.

Sprague-Dawley and Long-Evans rats do not differ in baseline open-field locomotion behaviors (i.e., horizontal activity and total distance, vertical activity, time spent in the center of the field) although the females of each strain are more active than are the males (Faraday & Grunberg, 1999; Faraday et al., 1999b).

Relatively few studies have examined effects of stress on locomotion. It has been reported, for example, that foot shock decreased horizontal activity in male Sprague-Dawleys



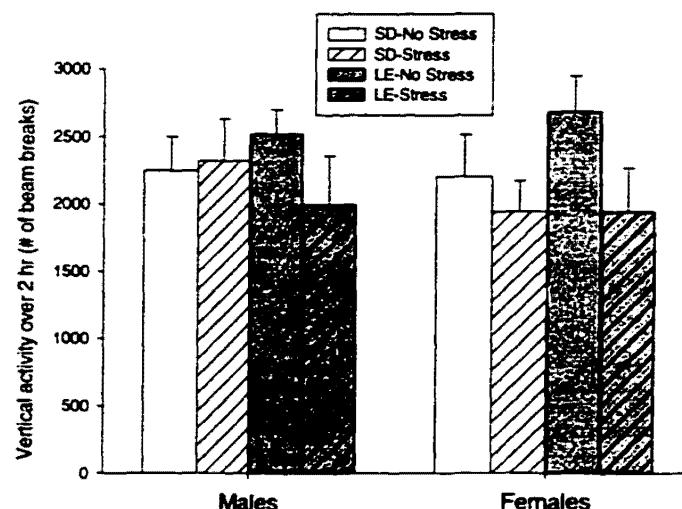
**Figure 4.** Center time on Day 4 of no stress or stress.



**Figure 5.** Center time on Day 10 of no stress or stress.

and that immobilization decreased horizontal activity in male Long-Evans

(Lemoine et al., 1990; Trudeau, Aragon, & Amit, 1990). In pilot work, locomotion responses (over 2 hrs) of male and female Sprague-Dawley and Long-Evans were measured after four and ten days of 20 min/day immobilization. Effects of immobilization depended on the sex and strain of rat, measurement day, and the variable measured (see Figures 4 - 6). On Day 4 (see Figure 4) stress decreased center time for Sprague-Dawley males but did not reliably alter locomotion behaviors of other groups. By Day 10 stress effects had disappeared in Sprague-Dawley males but were evident in Sprague-Dawley females as decreased center time (see Figure 5). Stress effects also were evident in Long-Evans females as decreased horizontal activity, decreased total distance, and decreased vertical activity (Figure 6). The lack of effects in Long-Evans males is not consistent with findings of Trudeau and colleagues (1990), however, subjects in that experiment were measured for only 10 min post-stress. These preliminary data are consistent with the idea that the two strains and sexes exhibit different behavioral coping responses to repeated mild stress.



**Figure 6.** Vertical activity on Day 10 of no stress or stress.

**Acoustic startle reflex (ASR) with and without pre-pulse inhibition (PPI).** The acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) of the ASR

are behavioral responses believed to index central processes related to information processing (Swerdlow, Caine, Braff, & Geyer, 1992) and possibly attention (Acri, Grunberg, & Morse, 1991; Acri, 1994; Acri et al., 1994, 1995; Grunberg, Acri, & Popke, 1994; Faraday et al., 1998, 1999a). ASR and PPI responses stabilize after three acclimation exposures to the testing situation, and animal responses remain relatively consistent after acclimation (e.g., Faraday & Grunberg, 2000). The ASR with and without a pre-pulse is included in the present experiment to assess reactivity to an acoustic stimulus (ASR) as well as sensory-gating or attention (PPI).

The acoustic startle reflex is a characteristic sequence of involuntary, defensive, muscular responses elicited by a sudden, intense acoustic stimulus (Davis, 1984). The reflex is present in all mammals, including humans and rats, and is considered an index of reactivity to external acoustic stimuli. Because the reflex can be elicited using the same stimuli across species (Swerdlow, Braff, Taaid, & Geyer, 1994), the paradigm has face validity for generalizing from an animal model to human responses. Although the neural pathway underlying the startle response is located largely in the brainstem (Davis, 1984), other non-brainstem structures can modulate startle responses, including the hippocampus, septum, periacqueductal gray, median raphe, and inferior colliculus (Caine, Geyer, & Swerdlow, 1992; Coover & Levine, 1972; Blair, Liran, Cytryniak, Shizgal, & Amit, 1978; Davis, 1984). Given the role of these structures in startle modulation, it is not surprising that the reflex also can be altered by higher level processes such as attention (Anthony & Graham, 1983;

Simons & Zelson, 1985) and emotion (Bradley, Cuthbert, & Lang, 1990).

Pre-pulse inhibition (PPI) of the acoustic startle reflex (ASR) occurs when the startling stimulus is preceded by a non-startling acoustic stimulus by a short interval (about 100 msec). The presence of the pre-pulse results in measurably reduced startle amplitude (Graham, 1975; Braff et al., 1978). This reduction in startle amplitude is pre-pulse inhibition of the ASR. As with the ASR, the phenomenon of pre-pulse inhibition occurs in humans and in rats. Pre-pulse inhibition is believed to index an innate sensory-cognitive-motor "gating" mechanism that operates at a non-volitional level and underlies the organism's ability to select relevant stimuli from the environment while screening out irrelevant information (Swerdlow et al., 1992). The circuitry underlying pre-pulse inhibition has not been completely elucidated but is complex, reflecting the integration of inputs from the hippocampus, amygdala, cingulate gyrus, ventral tegmental area and nucleus accumbens (Swerdlow et al., 1992).

There are strain differences in baseline, unmanipulated startle and PPI responses, with Sprague-Dawley rats exhibiting greater startle amplitudes and greater pre-pulse inhibition (PPI) than Long-Evans rats (Acri et al., 1995; Faraday et al., 1999a). Sex differences also exist within the two strains, with Long-Evans females startling less to louder stimuli than Long-Evans males but Long-Evans males exhibiting greater PPI than Long-Evans females (Faraday et al., 1999a). Among Sprague-Dawleys, males exhibit greater PPI than females (Faraday et al., 1999a).

Stress can alter startle and PPI separately or jointly, and whether stress

enhances, has no effect, or diminishes these responses when compared to non-stress controls depends on a number of factors, including the particular stressor used and the sex and strain of the animal. Whether a given stressor alters one or both parameters is relevant to data interpretation. An increase in reactivity (e.g., in startle amplitude) without a compensatory increase in gating processes (e.g., in percent pre-pulse inhibition) suggests that a stressor resulted in overall impaired gating. For example, cold and warm forced swimming in Sprague-Dawley males have been reported to have no effect on startle amplitudes but to decrease PPI, suggesting an overall impairment in sensory-gating post-stress (Leitner, 1989).

Effects of immobilization on ASR and PPI depend on subjects' sex and strain. Immobilization increased male Sprague-Dawley startle and PPI amount (Acri 1992, 1994; Faraday et al., 1999a), indicating that stress resulted in enhanced reactivity but also in compensatory, enhanced sensory-gating. In contrast, immobilization did not affect startle or PPI of female Sprague-Dawleys or of Long-Evans males (Faraday et al., 1999a). Further, immobilization decreased Long-Evans female startle and increased PPI, indicating that stress reduced reactivity but enhanced gating for these animals (Faraday et al., 1999a).

**Passive avoidance performance.** In the passive avoidance task, rats must learn to remain in a lit chamber, despite having access to a preferred dark chamber, in order to avoid a mild foot shock. The task can be used to study learning or acquisition of the correct response by subjecting animals to drug or stress treatments during the training procedure. It also can be used to assess

effects of manipulations on memory by testing animals' post-training performance after they undergo a drug or stress experience. In the present experiment, passive avoidance performance will be used to assess effects of stress on a simple memory task.

A literature search revealed no published studies on effects of immobilization stress on passive avoidance (PA) in Sprague-Dawley or Long-Evans rats. A few studies have examined effects of other stressors on PA in Wistar rats, another albino strain. These studies indicated that swim stress or tail shock prior to the training session improved memory performance (i.e., latencies of stressed animals to enter the dark chamber were longer than those of controls) (Kumar & Karanth, 1996; Pare, 1996).

***Morris water maze performance.*** In 1981, R.G.M. Morris demonstrated that rats could learn to locate an object that they could not see, hear, or smell as long as the object remained in a fixed spatial location relative to distal room cues (Morris, 1981). This task — the Morris water maze — consists of a large, round tub of water into which a platform (hidden or marked) is placed. The maze is used to test spatial learning in general, and reference memory vs. working memory in particular. In the present experiment, Morris water maze performance will be used as an index of complex cognitive functioning.

In reference memory versions of the maze, the platform is generally hidden just beneath the surface of the water, but is always located in the same place (e.g., Morris, 1981, 1984; Eichenbaum, Stewart, & Morris, 1990; Lindner, Balch, & VanderMaelen, 1992; Kraemer, Brown, Baldwin, & Scheff, 1996; Roof

& Havens, 1992; Hodges, Sowinski, Sinden, Netto, & Fletcher, 1995; Sandi, Loscertales, & Guaza, 1997). The animal must learn to navigate to the platform's location by using cues in the room. This version of the task is analogous to remembering where one's house is located.

In working memory versions of the task, the platform is either hidden or marked on Trial 1 and hidden on Trial 2, and is moved after each pair of trials (Morris, 1984; Morris, Hagan, & Rawlins, 1986; Lindner et al., 1992; Hodges et al., 1995). In this more difficult task, the animal must remember the most recent location of the platform (by using room cues) in order to correctly navigate to it on the second trial. This version of the task is analogous to remembering where one parked one's car today.

Male Sprague-Dawley and Long-Evans rats have been used extensively as subjects in studies of water-maze performance, and despite the visual differences between the strains, both perform well. Male Long-Evans rats, however, have been reported to perform better on Morris water maze tasks than male Sprague-Dawley rats (Tonkiss, Shultz, & Galler, 1992). Female Sprague-Dawleys also can perform the tasks (e.g., Rahman, 1999). The responses of Long-Evans females have not been examined.

A literature search did not reveal any studies on effects of stress on water-maze performance. It is known, however, that water maze performance depends on corticosteroid presence. For example, removal of endogenous corticosterone via adrenalectomy impairs performance on this task as does central administration of corticosteroid antagonists (Oitzl & de Kloet, 1992). In

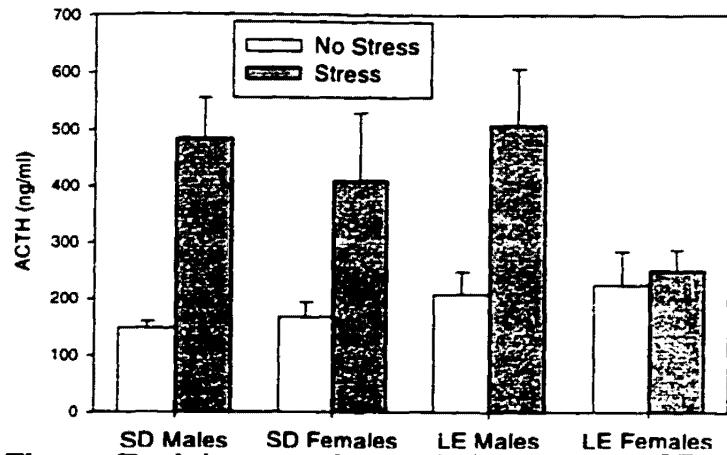
addition, a trend for a positive correlation between basal corticosterone levels and water maze performance has been reported (Yau, Olsson, Morris, Meaney, & Seckl, 1995). Increasing corticosteroid levels post-training by peripheral corticosteroid administration also has been reported to improve performance in male Wistar albino rats (Sandi et al., 1997), suggesting that moderate levels of stress might improve performance on this measure. Immobilization stress has been reported to improve performance in the radial-arm maze, another spatial memory task (Luine, Martinez, Villegas, Magarinos, & McEwen, 1996).

***Peripheral biochemical responses. Corticotropin releasing factor (CRF).*** Corticotropin-releasing factor is a peptide hormone made in the hypothalamus that stimulates the release of adrenocorticotropin hormone (ACTH) and beta-endorphin from the anterior lobe of the pituitary (Vale, Spiess, Rivier, & Rivier, 1981). In addition to its role in the HPA axis-mediated endocrine responses to stress, it is widely distributed in the central nervous system and produces a spectrum of autonomic, electrophysiological, and behavioral effects consistent with a neuromodulator role in the brain (Cummings, Elde, Ells, & Lindvall, 1983; Olschowka, O'Donohue, Mueller, & Jacobowitz, 1982; De Souza & Grigoriadis, 1995; Dunn & Berridge, 1990; Owens & Nemeroff, 1991). The highest concentrations of CRF-binding sites are in brain regions involved in cognitive function (cerebral cortex), in limbic areas involved in emotion and stress responses (amygdala, hippocampus, nucleus accumbens), and in brainstem regions regulating autonomic function (e.g., locus coeruleus) (De Souza, 1987, 1995). Because of its wide distribution, CRF has been described

as an integrator of brain, endocrine, and immune responses to physiological, psychological, and immunological stimuli (De Souza, 1995).

When administered centrally in rats, CRF activates

the sympathetic nervous system, resulting in increased plasma concentrations of catecholamines and glucose, increased heart rate and mean arterial blood pressure, and decreased gastrointestinal function (Brown & Fisher, 1990; Dunn & Berridge, 1990; Owens & Nemeroff, 1991). Behaviorally, CRF administration increases locomotion and rearing in familiar surroundings, increases agitation or emotionality in unfamiliar surroundings, potentiates acoustic startle, and decreases feeding (Dunn & Berridge, 1990; Kalin, 1990; Koob & Britton, 1990; Owens & Nemeroff, 1991; Swerdlow, Geyer, Vale, & Koob, 1986; Britton, Koob, River, & Vale, 1982; Morley & Levine, 1990). In humans, elevated CRF is associated with depression, anxiety, and anorexia nervosa (De Souza, 1990; Nemeroff et al., 1988; Owens & Nemeroff, 1991; Roy-Byrne et al., 1986; Gold et al., 1986). In the present experiment peripheral levels of CRF were measured as an index of HPA activation and of possible individual differences in HPA activation. CRF levels in the periphery are reported to vary in parallel with CRF levels in the hypothalamus (Yokoe et al., 1988).



**Figure 7. Adrenocorticotropin hormone (ACTH) on Day 14 of no stress or stress.**

*Adrenocorticotropin hormone (ACTH).* Adrenocorticotropin hormone is secreted from the anterior lobe of the pituitary in response to CRF stimulation and stimulates the adrenal cortices to manufacture and release corticosterone via second messenger cascades (Guyton, 1991). Unlike CRF, ACTH has not been demonstrated to exert other diffuse central neuromodulatory effects. ACTH was measured in the present experiment as an index of HPA activation and of possible individual differences in HPA activation to different stressors. Pilot work indicated that 14 days of immobilization stress increased ACTH levels in male and female Sprague-Dawleys and in male Long-Evans — an appropriate response to a mild stressor — but not in female Long-Evans (see Figure 7). These preliminary data suggest that female Long-Evans did not exhibit adaptive HPA axis responses to repeated presentation of a mild stressor.

*Corticosterone.* Corticosterone is the predominant 17-hydroxycorticoid made and released by the rat adrenal cortex in response to ACTH stimulation. Increases in corticosterone are widely used to index stress responses in rats, including responses to immobilization (e.g., Kant et al., 1983, 1987; Raygada et al., 1992; Shaham et al., 1992; Acri, 1994; Faraday & Grunberg, unpublished data). Corticosterone has powerful effects on protein, fat, and carbohydrate metabolism and acts at diverse sites in the periphery, including the immune system (spleen, thymus, lymph nodes), as well as in the brain (e.g., hypothalamus, pituitary, hippocampus, amygdala, cortex). In the brain, corticosteroid actions affect the neurochemistry of monoamines, peptide and amino acid transmitters, and processes of cell loss and damage, resulting in

changes in mood and behavior (McEwen et al., 1986).

Corticosteroid responses to stressors have been described as protecting the organism from the consequences of other, more rapid, stress responses (i.e., by reducing inflammation, maintaining blood glucose, and promoting fluid excretion) (McEwen et al., 1986). In the short-term, corticosteroid actions are adaptive and preserving and reflect healthy behavioral adjustments to stressors. However, long-term corticoid elevations are maladaptive and are associated with a variety of physical and psychological health problems (e.g., Rose, Jenkins, Hurst, Herd, & Hall, 1982; Rose, Jenkins, Hurst, Kreger, Barrett, & Hall, 1982; Rose, Jenkins, Hurst, Livingston, & Hall, 1982; Sapolsky, 1983; Carroll, 1977; Hanin et al., 1985). Corticosterone was measured in the present experiment as an index of HPA activation and of possible individual differences in HPA.

It is important to note that the catecholamines (i.e., epinephrine, norepinephrine) also would be relevant biochemical processes to measure in the present experiment. For example, excessive sympathetic activity is associated with certain health-threatening behavioral patterns (i.e., the hostile Type A pattern). In addition, human laboratory and field studies of stress have demonstrated the value of catecholamine measurement (e.g., Baum, Grunberg, & Singer, 1982; Davidson & Baum, 1986; Baum & Fleming, 1993; Frankenhaeuser, 1971, 1976, 1979; Frankenhaeuser, Lundberg, Fredrikson, & Melin, 1989). Further, dysregulation of central noradrenergic systems in particular has been implicated in hypertension, depression, and clinical anxiety states and it has been suggested that catecholamine phenotyping may be a

valuable approach for the diagnosis and treatment of some neurogenetic disorders (e.g., Goldstein, Lenders, Kaler, & Eisenhofer, 1996; Robertson et al., 1991).

The principal reason for not measuring catecholamines in the present experiment is logistical. Peripheral catecholamine responses occur within seconds and are provoked by many different environmental stimuli, including the handling and venipuncture necessary to obtain blood samples. It would be difficult to disentangle catecholamine responses associated with stressor presentation from those associated with the sampling procedure itself. One means of avoiding this confound is to fit animals with indwelling catheters so that samples can be withdrawn without disturbing the subject. Because of the many different behavioral measures proposed for use in the present experiment, this approach is not feasible. Future studies should be designed, however, specifically to examine the role of catecholamines in this rat model of individual differences. This issue is addressed further in the Discussion.

## HYPOTHESES

This experiment was an animal model of individual differences in behavioral and biologic stress responses that might be potential markers of specific stress vulnerabilities. The experiment was a 2 (Sprague-Dawley or Long-Evans) X 2 (male or female) X 2 (no stress or immobilization stress) full factorial design. The goals of the experiment were to: characterize stress responses in a rat model across a range of dependent variables within the same subject; determine the extent to which stress responses depend on the sex and strain of subject; and determine the extent to which a range of stress responses exists among these subgroups. A further goal of the experiment was to characterize patterns of stress responses in terms of their utility as markers for specific types of stress vulnerability.

There were seven major hypotheses that refer to the seven dependent measures: 1) body weight; 2) feeding; 3) locomotion; 4) ASR and PPI; 5) passive avoidance; 6) Morris water maze performance; and 7) HPA axis hormonal responses (hormonal responses are treated as a single dependent measure for the purpose of hypotheses). Potential markers of stress vulnerability were operationally defined as: over- or under-eating; marked gains or losses in body weight; decreases in activity and increases in anxiety indices (as revealed by different locomotion parameters); impairments in sensory-gating (as revealed by changes in startle with and without a pre-pulse); impairments in simple memory performance (indicated by passive avoidance performance); impairments in complex memory performance (indicated by Morris water maze performance);

and inappropriate HPA axis hormonal responses (either hypo-reactivity or hyper-reactivity). The degree of stress vulnerability was inferred by determining how many and to what degree these indices revealed maladaptive responses across and within specific subgroups.

**Hypothesis 1.** It was hypothesized that, in comparison to no stress controls, immobilization stress would reduce male body weight regardless of strain, would reduce Long-Evans female body weight, and would have no effect on Sprague-Dawley female body weight. This hypothesis was based on pilot work with Sprague-Dawley and Long-Evans males and females that used immobilization stress and obtained this pattern of results (Faraday et al., 1998).

**Hypothesis 2.** It was hypothesized that, in comparison to no stress controls, immobilization stress would reduce male feeding regardless of strain, would reduce Long-Evans female feeding, and would have no effect on Sprague-Dawley female feeding. This hypothesis was based on pilot work with Sprague-Dawley and Long-Evans males and females that used immobilization stress and obtained this pattern of results (Faraday et al., 1998) and on one published study using this stressor with Sprague-Dawley males and females (Zylan & Brown, 1996).

**Hypothesis 3.** It was hypothesized that effects of immobilization stress on locomotion would depend on the timing of measurement as well as on the strain and sex of subject. Specifically, it was hypothesized that, in comparison to no stress controls, immobilization stress would alter locomotion indices of

Sprague-Dawley males early in the experimental period (i.e., during the first week of stress), would alter locomotion indices of Sprague-Dawley and Long-Evans females late in the experimental period (i.e., during the second week of stress, and would not alter locomotion indices of Long-Evans males. This hypothesis was based on pilot work and the findings of Faraday et al. (1999b). Because this hypothesis involved four related locomotion variables (horizontal activity, total distance, vertical activity, and center time), it was tested with multivariate analyses of variance (MANOVA).

**Hypothesis 4.** It was hypothesized that effects of immobilization stress on ASR and PPI would depend on the strain and sex of subject. Specifically, it was hypothesized that, in comparison to no stress controls, immobilization stress would increase male Sprague-Dawley startle and increase PPI amount in compensation (i.e., percent PPI will not change), decrease Long-Evans female startle but increase PPI amount (i.e., percent PPI will increase), and have no effect on startle or PPI of female Sprague-Dawleys or male Long-Evans. This hypothesis was based on the findings of Acri (1992, 1994) and Faraday et al., (1999a) and constitutes a replication of those results. Because this hypothesis involves six related ASR and PPI variables (startle amplitude to 120 dB, startle amplitude to 110 dB, percent PPI to each stimulus when presented with a 68 dB prepulse as well as an 82 dB pre-pulse), it was tested with MANOVAs.

**Hypothesis 5.** It was hypothesized that, in comparison to no stress controls, immobilization stress would alter passive avoidance performance for all

groups. This hypothesis was based on reports in male Sprague-Dawley rats that stressors can improve passive avoidance performance (Kumar & Karanth, 1996; Pare, 1996). There have been no reports in females or in Long-Evans male rats, so this hypothesis had no specific directionality.

**Hypothesis 6.** It was hypothesized that, in comparison to no stress controls, immobilization stress would alter Morris water maze performance for all groups. This hypothesis was an extension of the rationale used to formulate Hypothesis 5 — that mild stress would alter cognitive performance. Like Hypothesis 5, specific directionality was not proposed as there is no existing literature.

**Hypothesis 7.** It was hypothesized that, in comparison to no stress controls, immobilization stress would increase HPA axis hormone levels (i.e., CRF, ACTH, and corticosterone) when compared to no-stress controls for all groups except for Long-Evans females. This hypothesis was based on the extensive work of others (e.g., Kant et al., 1983, 1987; Raygada et al., 1992; Shaham et al., 1992; Acri, 1994) as well as on pilot work indicating that after two weeks of daily stress exposure Sprague-Dawley males and females, and Long-Evans males showed an ACTH and corticosterone increase above non-stress controls but Long-Evans females did not. This hypothesis extended these findings to CRF because it is the biochemical stimulus for the production and release of ACTH. Because this hypothesis involves several related variables, it will be tested with MANOVAs.

## METHODS

### Overview

The experiment examined individual differences in behavioral and biologic responses of rats to repeated exposure to a mild physical stressor. Individual differences were examined in terms of Sex differences, Strain differences, and Sex X Strain interactions (i.e., differences at the level of same-sex, same-strain subgroups).

Some of the dependent variables — feeding, body weight, locomotion, acoustic startle with and without a pre-pulse — were measured several times over the course of the experiment. Repeated measurement over time allowed examination of short-term as well as chronic stress effects. In addition, repeated measurements allowed determination of responses that habituated to stress, responses that became sensitized to stress, responses that remained consistently altered by stress, and responses that remained unaffected by stress. Evidence that particular behaviors did not habituate to stress or became sensitized to stress, as well as individual differences in these patterns or in the time course of these patterns (i.e., strain differences, sex differences, within-strain and within-sex differences) was used to infer stress vulnerability. Because stress is omnipresent in human daily life, understanding the extent to which individual differences exist in these patterns is relevant to understand how stress alters performance, appetitive behaviors, and health, and how to alleviate damaging effects of stress.

Behavioral dependent variables that required training — passive avoidance performance and Morris water maze performance — were measured during training periods and during testing periods. Animals in the stress groups were trained and tested while also undergoing the relevant stress manipulations. This procedure allowed the examination of effects of stress on learning as well as on memory. Peripheral biochemical dependent variables were measured once, after sacrifice.

### **Experimental Design and Determination of Sample Size**

The experiment was a 2 (Long-Evans or Sprague-Dawley) X 2 (male or female) X 2 (no stress or immobilization stress) full factorial design with about 20 subjects per cell. This sample size was selected to optimize statistical power across a range of dependent measures that vary in effect size in response to mild stressors and was determined according to three criteria:

(1) Previous studies and pilot work that used many of the same dependent measures [i.e., feeding, body weight, locomotion, acoustic startle reflex (ASR) and pre-pulse inhibition (PPI), corticosterone, and adrenocorticotropin hormone (ACTH)] with 10 subjects per cell were used as a starting point.

(2) Because effects of immobilization stress on body weight, ACTH, and corticosterone were robust in pilot data but effects on locomotion, ASR and PPI, and feeding were not as clearly demonstrated (e.g., statistical trends), power analyses were performed to determine the probability of correctly detecting an effect and to corroborate sample size.

Analyses were conducted using procedures of Keppel (1991), Keppel, Saufley, and Tokunaga (1992), and Cohen (1988). Estimates of effect size in the population were determined by calculating an estimated omega squared ( $\omega^2$ ) according to the formula:

$$\omega^2_A = \frac{\sigma^2_A}{(\sigma^2_A + \sigma^2_{S/A})}$$

where  $\sigma^2_A$  refers to the estimated population treatment effects and  $\sigma^2_{S/A}$  refers to the estimated population error variance (Keppel et al., 1992, p. 180). The omega squared statistic provides a measure of effect size that is relatively independent of sample size and is expressed as a proportion of the total variability ( $\sigma^2_A + \sigma^2_{S/A}$ ) that is associated with the treatment or manipulation ( $\sigma^2_A$ ). A value for the phi statistic ( $\phi$ ) was then calculated according to the formula:

$$n = \phi^2 \left( \frac{1 - \omega^2_A}{\omega^2_A} \right)$$

(Keppel et al., 1992, p. 213). The phi statistic indicates the ratio of treatment variance to error variance for a given sample size (Keppel, 1991, p. 77). Using phi and the appropriate power function in Table A-6<sup>2</sup> (Keppel et al., 1992, p. 538), power was determined. These calculations on data obtained using 10

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<sup>2</sup>

Table A-6 contains power functions taken from E.S. Pearson and H.O. Hartley (Eds.), *Biometrika Tables for Statisticians* (Vol. II), Cambridge University Press, London, 1972, and from J. Rotton and P.S. Schonemann, Power tables for analysis of variance, *Journal of Educational and Psychological Measurement*, 1978, 38, pp. 213-229.

subjects per cell revealed a mean power of 0.60 across measures.

The same formulas were then used to calculate the  $n$  per cell necessary in order to achieve power of 0.80 across measures as recommended by Cohen (1988). These calculations revealed that for measures with the smallest effect size (locomotion, ASR and PPI, feeding) approximately 15-16 subjects per cell were necessary.

(3) There are no published studies on effects of immobilization stress on passive avoidance or Morris water maze performance. However, experience in our laboratory using these measures indicates that moderate manipulations (e.g., effects of prenatal drug exposure on subsequent offspring performance) are difficult to detect with an  $n$  of 10 per cell. Because effects of mild physical and psychological stressors are expected to be moderate on these measures as compared to effects of more powerful manipulations, such as brain lesioning, that are commonly evaluated in these paradigms (and statistically detectable with 6 to 10 subjects per cell), an increased sample size seemed warranted.

Therefore, an  $n$  per cell of 16 animals was selected. In order to validate the stressor halfway through the Stress Phase, four subjects were added to each cell (bringing the  $n$  per cell to 20). These subjects were sacrificed on Stress Day 11 and trunk blood samples were collected for biochemical assays. Because the animal breeder provided several extra animals in the shipment and because additional animals were needed to have sufficient subjects for the purposes of a collaboration that used samples from this dissertation but was not proposed as part of it, the final  $N$  was 167.

## Subjects

Subjects were 87 adult Sprague-Dawley rats (42 male, 45 female) and 80 adult Long-Evans rats (40 male, 40 female) (Charles River Laboratories, Wilmington, MA) about 60 days old at the beginning of the experiment. This subject age was chosen because rats undergo sexual maturation between days 50 and 60 (P. Mirley, Charles River, personal communication, 8/15/98) and the goal of the present experiment was to examine behavioral and biological responses to stress in fully adult animals.

Animals were housed individually throughout the experiment in standard polycarbonate shoebox cages (42 x 20.5 x 20 cm) on hardwood chip bedding (Pine-Dri). Individual housing was used so that the feeding responses of each animal could be measured. It has been reported that among Wistar rats individual housing is stressful for females as indexed by corticosterone levels (levels > 600 ng/ml) (Brown & Grunberg, 1995). Corticosterone levels measured in individually-housed or group-housed pilot animals suggest, however, that this effect varies with rat strain. In particular, after six weeks of the individual housing condition, Sprague-Dawley females had a mean corticosterone level of 368.9 ng/ml (Sprague-Dawley males had mean levels of 246.8 ng/ml) and Long-Evans females had a mean corticosterone level of 522.0 ng/ml (Long-Evans males had mean levels of 206.8 ng/ml). It is not clear whether this relatively high level of corticosterone in individually-housed Long-Evans females reflects a stressed state, however, because a separate study revealed that Long-Evans females housed in same-sex groups of six had even higher average corticosterone levels

of 718.8 ng/ml. Therefore, the individual housing condition appears to result in the lowest female corticosterone levels for the rat strains used in this experiment.

Throughout the study subjects had continuous access to rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Housing rooms were maintained at 23° C at 50% relative humidity on a 12-hour reversed light/dark cycle (lights on at 1800 h). A reversed light cycle was used so that rats would be in their active phase (the dark phase) during the day, allowing behavioral assessments to be made during working hours. Sacrifices took place during the light part of the cycle, when HPA axis hormones are at the low point in the circadian cycle, in order to maximize hormonal differences between non-stressed and stressed animals.

### **Timetable**

The experiment proceeded in three phases: **Baseline Phase, Stress Phase, and Biochemistry Phase**. Table 1 below presents the timeline for the **Baseline and Stress Phases**.

During the **Baseline Phase**, animals were gentled, feeding and body weight measurements began, animals were acclimated to the locomotion apparatus and to the acoustic startle apparatus, and baseline locomotion and ASR and PPI measurements were obtained. Gentling consisted of handling each animal for two min for two consecutive days in order to accustom subjects to routine handling (i.e., moving animals from the home cage into the equipment and back to the home cage) that is necessary for behavioral testing and body

<b>BASELINE PHASE</b>	
<b>Day of Experiment</b>	<b>Procedure/Dependent Measure</b>
1	Rats arrive
2 - 3	Gentling
3 - 7	ASR and locomotor acclimation
8 - 9	ASR and locomotor BASELINES
<b>STRESS PHASE</b>	
1	Locomotion
2	ASR and PPI
4	Passive avoidance training
5	Passive avoidance testing
6	Locomotion
7	ASR and PPI
9	Locomotion
10	ASR and PPI
11	Sacrifice of 4 animals per cell
12-18	Morris water maze training/testing
19	Locomotion
20	ASR and PPI
22	Sacrifice of remaining animals

Table 1: Experimental Timeline

weight measurement. Acclimation was done in order to minimize the effects of possible stress as a result of exposure to a novel situation or apparatus.

Acclimation involved placing the animal in the apparatus for the same amount of time that would later be necessary during Baseline and Stress phase measurements. Two acclimation exposures were provided to the locomotion

apparatus before baseline data collection. Four exposures to the ASR apparatus (one exposure to the equipment without presentation of the acoustic stimuli and three exposures to the complete testing procedure) were conducted before baseline data collection. These acclimation procedures have been empirically determined to result in stable behavioral baselines (Faraday & Grunberg, unpublished data; Faraday & Grunberg, 2000). The **Baseline Phase** lasted approximately 9 days. At the end of this phase, subjects were assigned within-strain and within-sex to the no stress or immobilization stress groups in a manner that ensured comparable initial mean body weights and comparable locomotor activity among same-strain, same-sex groups.

During the **Stress Phase**, feeding and body weight measurements were made every other day. On Stress Day 1, stress exposure began for animals in all of the stress groups such that approximately 30 min before each scheduled behavioral measure, animals in the stress groups underwent 20 min of immobilization stress.

During this period locomotion and ASR and PPI were measured several times. Specifically, locomotion was measured on Stress Days 1, 6, 9, and 19; ASR and PPI were measured on Stress Days 2, 7, 10, and 20. During this phase, each animal also underwent passive avoidance training on Stress Day 4 and passive avoidance testing 24 hr after training on Stress Day 5. On Stress Day 11 four animals from each cell were sacrificed. From Stress Day 12 through 18, the remaining animals were trained to perform the Morris water maze task as

described in detail below. Animals were sacrificed on Stress Day 22. Prior to sacrifice, animals in the stress groups underwent 20 min of immobilization stress. The Stress Phase lasted 22 days.

The Biochemistry Phase consisted of assays on samples obtained at the sacrifices. Assays were conducted according to procedures described below.

#### **Stress Manipulation: Immobilization**

Animals in the immobilization stress condition were restrained in commercially available finger-like restraining devices (Centrap Cage, Fisher Scientific) 20 min/day during the Stress Phase. Subjects were placed in the Centrap cage and the restraining "fingers" were tightened until subjects were immobilized, but not pinched or in apparent pain. Restrained animals were checked every 5 min during the stress procedure to insure the manipulation did not result in pain (as indicated by vocalizations). This restraint procedure has reliably produced elevations in hormones associated with a stress response, including adrenocorticotropin hormone (ACTH) and corticosterone (e.g., Acri, 1994; Raygada et al., 1992; Kant et al., 1987, 1983; Faraday & Grunberg, unpublished data).

#### **Dependent Variables**

**Feeding and body weight.** Feeding and body weight were measured throughout the experiment using Sartorius electronic balances. Balances were programmed to determine body weight by computing the mean of ten weighings taken within 2 sec. This procedure controlled for animal movement artifacts and

provided an accurate body weight assessment. Food consumption also was measured using Sartorius balances.

**Locomotion.** Locomotor activity was measured using an Omnitech Electronics Digiscan infrared photocell system [Test box model RXYZCM (16 TAO); Omnitech Electronics, Columbus, OH], located in a dedicated room within the animal facility. This room is constructed of cinderblock walls, acoustic tile ceiling, and steel doors so that sound is kept to a minimum. Two hour activity measurements were obtained during animals' active or dark cycle. Dark cycle measurement was done because animals' baseline level of activity during this part of the circadian cycle is sufficiently high to allow measurement of activity decrements and also low enough that activity increases can be reliably measured. Animals were placed singly in a 40 X 40 X 30 cm clear Plexiglas arena and a Plexiglas lid with multiple 3.5 cm diameter holes was placed on top of the arena. The lid ensured that subjects had adequate ventilation but could not escape during data collection. A photocell array measured horizontal locomotor activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2 cm above the floor of the arena. A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measured vertical activity. Data were automatically gathered and transmitted to a computer via an Omnitech Model DCM-I-BBU analyzer. Once subjects were placed in the test arenas, the experimenter turned off the lights and left the room.

The interfaced software generates 21 subvariables, including total

distance in cm (a measure of overall activity), horizontal and vertical activity (measures of activity in the horizontal plane and exploratory activity, respectively), and time spent in the center vs. the margin of the open field (a possible measure of anxiolysis). These four subvariables were analyzed as indices of arousal/general activity, exploration, and possible anxiety, respectively.

**Acoustic startle reflex (ASR) with and without pre-pulse inhibition (PPI).** Acoustic startle reflex amplitudes and pre-pulse inhibition were measured in a Med Associates Acoustic Response Test System (Med Associates, Georgia, VT). The Acoustic Response Test System consists of weight-sensitive platforms inside individual sound-attenuated chambers. Subjects' movements in response to stimuli were measured as a voltage change by a strain gauge inside each platform. Responses were recorded by an interfaced Pentium computer as the maximum response occurring during the no-stimulus periods, during the pre-pulse period, and during the startle period.

Each rat was individually placed in a ventilated holding cage. The holding cages are small enough to restrict extensive locomotion but large enough to allow the subject to turn around and make other small movements. Each cage was then placed on a weight-sensitive platform. A ventilating fan built into the chamber provided background noise. Following placement of animals in the chambers, a 3-minute adaptation period ensued in which no startle stimuli were presented.

ASR and PPI responses were measured during the dark portion of the

circadian cycle because startle amplitudes are more stable at this time (Davis & Sollberger, 1971) and are greater during the dark cycle (Chabot & Taylor, 1992). Specifically, startle amplitudes increase by up to 100% during the dark portion of the daily cycle over mean startle values measured during the light portion (Chabot & Taylor, 1992). In addition, testing of subjects' responses when they are awake and alert provides face validity because findings will be used to extrapolate to responses of awake and alert humans.

Startle stimuli consisted of 110 or 120 dB noise bursts of 20 msec duration sometimes preceded 100 msec by 68 or 82 dB 1kHz pure tones (pre-pulses). These stimuli were selected because stimuli in this range are widely used in the literature (e.g., Harty & Davis, 1983; Caine et al., 1992; Swerdlow et al., 1993; Bakshi & Geyer, 1995) and softer stimuli (i.e., 100 dB) may not elicit a startle response in some animals (Faraday & Grunberg, unpublished data). Two pre-pulse levels were used in order to ensure comparability with prior experiments (Acri et al., 1991, 1995; Acri, 1994; Faraday et al., 1998, 1999a) and to clarify possible issues of interpretation. More specifically, past experiments conducted in this laboratory used a system (Coulbourn Instruments) with a 56 dB background noise level generated by a fan and a 68 dB pre-pulse. The Med Associates system was newly acquired and this experiment was the first experiment conducted using it. In contrast to the Coulbourn system, the Med Associates system fan generates a background noise level of 78 dB. Pilot work revealed that rats inhibited startle robustly to the 68 dB pre-pulse even though it was softer than the fan dB level. This inhibition presumably occurred

because the pre-pulse pure tone was clearly audible to the rats (as it was to the experimenter) against the background white noise of the fan. It is more common in the literature, however, to use a pre-pulse that is above background noise. Therefore, to ensure comparability with past work in this laboratory (using the 68 dB pre-pulse) and to address potential difficulties of interpretation with a pre-pulse below background noise, the stimuli were run with both pre-pulses.

Decibel levels were verified by a Larson-Davis Sound Pressure Machine Model 2800 (unweighted scale; re: 0.0002 dynes/cm<sup>2</sup>). Each startle stimulus had a 0 msec rise and decay time so that onset and offset were abrupt, a primary criterion for startle. There were six types of stimulus trials, and each trial type was presented eight times, for a total of 48 trials. Trial types were presented in random order to avoid order effects and habituation. Inter-trial intervals ranged randomly from 20 - 40 sec. Trial types included: (1) 120 dB stimulus, (2) 120 dB stimulus preceded by a 68 dB pre-pulse, (3) 110 dB stimulus, (4) 110 dB stimulus preceded by a 68 dB pre-pulse, (5) 120 dB stimulus preceded by an 82 dB pre-pulse, and (6) 110 dB stimulus preceded by an 82 dB pre-pulse.

The testing period lasted approximately 28 min. Holding cages were washed with warm water and dried after each use. Each sound-attenuated chamber was allowed to ventilate with the door open for 20 min between animals to optimize clearance of any pheromones that might be present in the chamber. Non-stressed and stressed subjects were tested in separate runs in a counterbalanced manner.

Recordings of subject movements during no-stimulus periods were used

to control for movements on the platform not related to the startle stimulus. Recordings of subject movements during pre-pulse periods were included to ensure that the pre-pulse tone itself did not elicit a startle response. For data analysis purposes, each animal's responses were averaged within trial type. Pre-pulse amounts were calculated by subtracting amplitude to each stimulus with a pre-pulse from amplitude to the same stimulus without pre-pulse. The remainder was analyzed as pre-pulse inhibition amount. Percent pre-pulse (PPP) was calculated as [(amplitude of trial without pre-pulse) - (amplitude of trial with pre-pulse)/amplitude of trial without pre-pulse] X 100. The product was analyzed as PPP. These calculations are based on established procedures of several investigators (Acri, 1994; Acri et al., 1995; Faraday et al., 1998, 1999a; Swerdlow, Caine, Braff, & Geyer, 1992; Swerdlow, Mansbach, Geyer, Pulvirenti, Koob, & Braff, 1990; Swerdlow, Vaccarino, Amalric, & Koob, 1986).

#### **Passive avoidance shuttlebox performance.**

**Apparatus.** Animals were trained and tested using an automated avoidance training system (Gemini, San Diego Instruments, San Diego, CA). The apparatus consists of two 21 x 25 x 17 cm chambers separated by a vertically-sliding door. Lighting in the chambers was provided by a 50 watt bulb 3 cm above the translucent ceiling. Scrambled, constant-current shocks were delivered through a grid floor. Control of the door, lighting, and shock are provided by means of a 486 personal computer running software (PA, San Diego Instruments, San Diego, CA).

**Procedure.** Training and testing procedures were similar. During training, the animal was placed in one chamber of the darkened apparatus. After a delay of 60 s, the 50 watt light came on in the chamber in which the rat had been placed and the door to the other, still darkened chamber, opened. Rats generally will move from the lit chamber into the dark chamber in less than 60 sec on this first exposure to the apparatus. When the rat crossed completely into the darkened chamber, the door closed, latency to cross was recorded by the interfaced computer, and a 0.8 mA shock was delivered for 1 sec. The rat was left in the darkened chamber in which the shock had been delivered for 30 sec, and then removed. If the rat did not cross into the darkened chamber, then it was removed after 300 s.

The testing procedure was identical except that shock was not delivered if the animal crossed into the darkened chamber. Memory is presumed to have occurred if the animal does not cross into the chamber in which it previously was shocked, or if latency to cross is statistically significantly longer during the testing trial than during the training trial. Testing was carried out 24 hours after training. Both chambers were cleaned with a 50% ethanol solution after each subject. On both days, all of the non-stressed subjects were run in the apparatus and then all of the stressed subjects were run. This procedure was followed in order to avoid exposing non-stressed subjects to pheromones of stressed subjects that might be present in the enclosed spaces of the shuttlebox compartments.

Because rodents placed in a novel environment instinctually move into darkened areas, greater than 90% of rats will cross into the still-darkened

chamber during the training procedure. A small percentage of animals will not cross, do not receive a shock, and, therefore, are not trained. These subjects (three animals of the total N of 167 in the present experiment, or 1.8%; one Sprague-Dawley non-stress female, one Long-Evans non-stress female, and one Long-Evans stress female) were re-exposed to the apparatus during the testing procedure so that all subjects had the same number of shuttlebox exposures, but data from untrained subjects were not included in the analyses.

**Morris water maze performance.**

**Apparatus.** The water maze apparatus consists of a circular, dark blue plastic tank 96 cm in diameter (72 cm in diameter at water level) and 50 cm high filled with 30°C ( $\pm 1$  °C) water. Water temperature is a critical variable in water maze methodology because cold water temperatures impair performance (e.g., water temperatures of 15 or 20°C) and can be used to introduce additional stress into the task (e.g., Sandi et al., 1997). This temperature was selected based on work by other investigators (Morris, 1981; Morris et al., 1986; Decker et al., 1993), past work in our laboratory (Rahman, 1999), and pilot work by the experimenter to provide a swimming temperature that did not produce signs of hypothermia, such as piloerection, or hyperthermia, such as panting.

The platform is a black acrylic structure that is placed in the pool and can be presented in a marked or in a hidden configuration. In the marked configuration, a white box with a gray textured surface (to facilitate animals climbing on to it) that extends above the surface of the water by 2.5 cm and below the surface by 10 cm is placed on top of the platform, making it visible to

the rat. In the hidden configuration, the white box is not used and the surface of the black escape platform remains 1.5 cm below the water surface — deep enough not to be seen or cause ripples from surface agitation, but shallow enough so that the rat will be out of the water once it finds the platform. In the present experiment the platform was used in both configurations (see **Procedure** below). To ensure that rats could not see the platform beneath the water surface, black nontoxic water-soluble tempera paint (either DryTemp, Palmer Paint Products, Inc., Troy, Michigan, or BesTemp, Certified Color Corp., Santa Ana, CA) was added to the water to make it opaque following the procedures of several investigators (Kraemer et al., 1996). Paint was added until the experimenter could no longer see the platform on the video screen or by looking in the pool. Although this concentration of paint was sufficient to make the water opaque, it did not leave any color residue on the animals' coats.

The maze is conceptually divided into 4 equal quadrants. The platform was always placed in the center of a particular quadrant, and on a given day, the platform was in the same location for all subjects. Platform locations were changed each day in a counterbalanced manner.

Because water maze performance depends, in part, on the animal's ability to use visuospatial cues in the environment, the maze was placed in a room with two types of extra-maze visual cues: large extra-maze objects and luminosity gradients. Extra-maze visual cues and luminosity gradients across the maze space are believed to provide spatial information critical for accurate maze performance (Kraemer et al., 1996). Large extra-maze objects consisted of

experimental equipment, black-and-white squares of different patterns placed on two walls adjacent to the maze, the presence of walls on two sides of the maze, and the presence of a large black door in one of the walls. A luminosity gradient was created by placing spotlights around the maze such that the "south" (arbitrary designation) wall was brightly lit, the "east" wall was moderately lit, and the "north" and "west" walls will be open to the rest of the room but dimly lit. It has been suggested that, for rats, luminosity gradients are functionally superior cues to those more extensively used by humans, such as the position of objects in a room (Olton, 1977). Other evidence supports the idea that even pigmented rats use visual information differently than humans. For example, when rats must distinguish between a "safe" platform that allows escape from the water and an unstable platform that does not allow escape on the basis of differential platform appearance alone, pigmented animals (i.e., Lister rats) require an average of 120 trials to reliably perform the discrimination (Morris, 1984). In addition, luminosity gradients may be particularly important for Sprague-Dawley rats because of poor visual acuity.

Each maze session was recorded using a computerized video tracking system (Polytrack System, San Diego Inst., San Diego, CA). This system consisted of a video camera mounted above the maze connected to a computer interface that tracked the moving rat in the pool. Data were automatically recorded and included latency to find the platform, total path length, and distance swum in each quadrant of the maze (the distance variables were included because they are not confounded by possible differences in swimming speed).

The camera and accompanying software are designed to track a white object against a dark background or a dark object against a white background. Addition of black paint to the water made the white Sprague-Dawley rats visible to the camera. Because the black and white Long-Evans rats had white markings that came up to the shoulder area, the camera also was able to detect these animals but with less consistency (i.e., depending on a specific animal's particular pattern of markings and on the animal's constantly-changing swimming angle to the camera, the camera periodically "lost" the animal and the track did not reflect the animal's complete path). In order to render the Long-Evans rats consistently visible to the camera, a 1.5 X 1.5 cm piece of white reflective tape was attached just above the shoulder blades with small piece of masking tape. Masking tape was used because it does not adhere well to fur, especially once wet, and could be removed without pulling out fur or causing discomfort to the animal. This procedure was tested with pilot animals and did not appear to interfere with animals' swimming ability. To the experimenter's observation, the animals generally did not appear to be aware of the tape. In addition, the manufacturer of the maze and tracking software (San Diego Instruments) recommended the use of reflective tape when problems with consistent tracking arose.

Ideally, reflective tape also would have been used with the Sprague-Dawley rats. The solution to the tracking problem described above was not determined, however, until after the Sprague-Dawleys had been run in the maze (because of the experimental logistics). An alternative solution that would not

have required use of tape on the Long-Evans rats was to set the tracking software to track a dark object against a light background. This alternative would have required several other procedural changes, however, including: 1) painting the dark blue maze white; 2) adding chalk or powdered milk to the water instead of black paint; and 3) changing the position of the lights to deal with a different pattern of reflections off the water. These changes would have resulted in a maze that was substantially different in appearance (i.e., different contrast properties, different visual cues, and altered luminosity gradients) from the maze used with the Sprague-Dawley rats. Because the use of tape allowed the Long-Evans rats to be run in the identical maze as that used for the Sprague-Dawleys without changing lighting or properties of the water, because this solution involved one procedural change as opposed to three changes, and because the experimenter's observation was that the animals were untroubled by the presence of the tape, this alternative was selected as the best choice for minimizing error variance in the circumstances.

In order to prevent home cages from becoming wet, after each trial animals were placed in a standard shoebox cage that contained a dry towel for approximately 10 min. During this period animals shook most of the water from their coats and groomed vigorously so that when they were returned to the home cage their fur was mostly dry. Different cages and towels were used for males and females and for non-stressed and stressed rats.

**Procedure.** The water maze task in the present experiment was a spatial learning and working memory task based on the classic procedures of

Morris (e.g., Morris, 1984, Morris et al., 1986), the originator of the maze, and of several other investigators (Lindner et al., 1992; Hodges et al., 1995) in which the location of the platform was changed after each pair of trials. Because minimizing stress as a result of exposure to a novel situation was critical to the present experiment and because the rat strains differed in visual acuity, several additional procedures were employed before the spatial and working memory task began to control for possible sources of experimental error.

Each animal received two maze trials per day for seven days for a total of 14 trials with a maximum trial swimming time of two minutes. Trials 1 through 4 (conducted on Days 1 and 2) were used to acclimate animals to the apparatus and procedures (i.e., to familiarize the rats with the presence of the platform in the water), to accustom them to swimming, and to rule out possible visual or motivational differences that might affect performance. Trials 5 through 14 (conducted on Days 3 through 7) constituted the spatial and working memory portion of the task.

More specifically, on Day 1 the platform was placed in the pool in the marked configuration. For Trial 1, each animal was released gently into the water facing the wall of the pool in the quadrant furthest from the platform. If the animal had not climbed onto the visible platform after two minutes of swimming, then it was guided to the platform and assisted onto it. The animal was left on the platform for 30 sec. Trial 2 began after an intertrial interval (ITI) of 30 min, and followed exactly the same procedures. Because these trials were primarily used for acclimation, this ITI was selected for logistical reasons.

The marked configuration was used for these initial trials in order to determine whether differences in visual acuity between unpigmented Sprague-Dawley rats and pigmented Long-Evans rats affected performance when the platform could be seen. Swimming to the marked platform is not a spatial task *per se* (because no spatial information is required to swim to a marked target), but may indicate whether differences in visual abilities are relevant to performance when the platform is hidden. For example, if Sprague-Dawley rats generally take longer and swim longer distances than Long-Evans rats to find the hidden platform but perform similarly to Long-Evans rats when the platform is marked, then performance differences when the platform is hidden are unlikely to be a result of visual differences.

Because maze performance depends, in part, on the animal's motivation to get out of the water, trials with the marked platform also can be used to rule out motivational differences that might affect performance (Morris & Schenk, 1983; Morris, 1984). For example, if certain subgroups (e.g., males vs. females, stressed vs. non-stressed animals) take longer and swim longer distances to reach the marked platform, differences in motivation to leave the water might be inferred. These differences, if they are revealed, can be controlled for statistically (i.e., by using performance when the platform is visible as a covariate and by analyzing subgroups separately).

On Day 2, the platform location was changed. On the first trial of the day (Trial 3), the platform was presented in the marked configuration and again each rat was released facing the pool wall from the quadrant furthest from the

platform. Animals were allowed to swim until they climbed onto the platform or until two min passed. Animals that did not climb on the platform after two min were gently guided to it. Once on the platform, animals were allowed to remain there for 30 sec and then removed. Trial 4 began after an intertrial interval of one hour. For Trial 4 the platform was in the same quadrant as in Trial 3, but was presented in the hidden configuration (i.e., the white box portion was removed so that the platform surface was hidden beneath the water). Rats were released facing the pool wall from a different quadrant than that used in Trial 3. Animals were allowed two minutes to find the hidden platform and those that did not find it were guided to it. The purpose of these two trials was to continue acclimating animals to the procedures, and to familiarize the animals with the fact that the platform was present even when it could not be seen and that it remained in the location from the previous trial regardless of release quadrant.

The one hour ITI used between Trials 3 and 4 and for all subsequent trials (Trials 5 through 14) was selected to maximize sensitivity of the task because the performance of young adult rats does not decline appreciably until the ITI is increased beyond one hour (Panakhova, Buresova, & Bures, 1984; Morris et al., 1986; Lindner et al., 1992). Longer ITIs, therefore, may reveal subtle cognitive deficits that may not be evident with shorter ITIs (e.g., Bartus, 1986; Goodrick, 1973; Thompson & Fitzsimons, 1976) and are appropriate when the manipulation (i.e., mild stress) or other independent variables (i.e., sex, strain) are expected to result in subtle behavioral changes.

On Days 3 through 7 the platform was hidden for all trials (trials 5 through

14). This portion of the experiment constituted the spatial learning and working memory task. The animal's task on each day was to find the platform on the first trial of the day, remember where it was for the one hour ITI, and use that information to swim more directly and quickly to the platform on the second trial of the day from a different starting point. On the first trial of each day, rats were placed in the pool facing the wall in a quadrant in which the platform was not located (counterbalanced over the course of the experiment) and allowed to search for the platform. Animals were allowed two minutes to find the hidden platform and those that did not find it were guided to it. Animals remained on the platform for 30 sec. One hour later rats were placed in the pool in a different quadrant (counterbalanced over the course of the experiment) and again allowed to search for the platform for two minutes. Animals that did not find the platform were guided to it. Animals again remained on the platform for 30 sec. The platform location was changed every day (also in a counterbalanced manner).

**Peripheral biochemical measurements. Corticosterone.** Total serum corticosterone was measured by a double-antibody radioimmunoassay (RIA) kit using <sup>125</sup>I-labeled corticosterone (ICN Biomedicals, Costa Mesa, CA). Specifically, a limited amount of specific antibody (rabbit anti-corticosterone antiserum) was reacted with a fixed quantity of <sup>125</sup>I-labeled corticosterone. The concentration of unlabeled corticosterone in samples increases as a function of the decreasing percentage of bound radioisotope-labeled corticosterone. The second antibody (goat anti-rabbit gamma globulin) precipitated antibody bound to antigen. The quantity of endogenous corticosterone was then determined by

measuring the radioactivity of the precipitate with known standards from the same assay in a gamma counter and converting DPM into concentrations. All samples and standards were run in duplicate. The sensitivity of the assay is 8 ng/ml (R. Gilmartin, ICN Biomedicals, 10/14/99). The coefficient of variation is 6.93%.

***Adrenocorticotropin hormone (ACTH).*** This assay is similar in principle and operation to the corticosterone assay described above. The only difference is that the assay required that samples be treated at collection with an anti-coagulant (15% EDTA; 1 mg per 1 ml whole blood) and a protease-inhibitor (aprotinin; 1,000 KIU per ml of whole blood). All samples and standards were run in duplicate. The sensitivity of the assay is 6 pg/ml (R. Gilmartin, ICN Biomedicals, 10/14/99). The coefficient of variation is 6.20%.

***Corticotropin-releasing factor (CRF).*** As with the ACTH sample collection, CRF sample collection required use of EDTA and aprotinin as described above. In addition, because peripheral concentrations of CRF are low, a reverse-phase extraction was performed on plasma samples to remove interfering substances and concentrate the CRF. Samples were acidified with equal amounts of 1% trifluoroacetic acid (TFA; HPLC grade) in order to remove interfering proteins such as albumin. Sep-columns containing 200 mg of C<sub>18</sub> (Peninsula Laboratories, Inc., Belmont, CA) then were equilibrated by washing once with 60% acetonitrile in 1% TFA (1 ml) and washing three times with 1% TFA (3 ml for each wash). Plasma solutions then were loaded onto the columns and the columns were washed twice with 1% TFA (3 ml for each wash). The

peptide was eluted by washing once with 60% acetonitrile in 1% TFA (3 ml). The eluant was collected in polypropylene tubes and quickly frozen by placing the tubes into a dry ice and ethanol bath. Frozen samples then were lyophilized in a Virtis Consol 12 lyophilizer (Virtis Co., Gardiner, NY) for 48 hours at 0°C and 10 millitorr to evaporate all moisture from the samples. Samples were stored at -80°C until assayed.

The radioimmunoassay is similar in principle and operation to the corticosterone assay described above. All samples and standards were run in duplicate. The sensitivity of the assay is 1 pg/ml.

## DATA ANALYTIC STRATEGY

The goals of data analysis were to determine: whether rat strain, sex, and/or stress altered each dependent measure; the extent to which the magnitude of stress effects varied among subgroups; and whether the relationships between or among variables differed based on the strain, sex, and stress status of subjects. Analytic approaches varied depending on properties of specific data sets (i.e., the number of times the given variable was measured, whether the variable met parametric test criteria). Details about specific analytic strategies are presented at the beginning of each dependent variable section.

In general, body weight and feeding data were analyzed with repeated-measures analyses of variance (ANOVAs) and ANOVAs on each measurement day. Locomotion dependent measures (total distance, horizontal activity, vertical activity, center time) were analyzed using multivariate analyses of variance (MANOVAs) because these variables are correlated. Acoustic startle amplitudes with and without a pre-pulse and percent pre-pulse inhibition also were analyzed using MANOVAs for the same reason. Passive avoidance data were analyzed with ANOVAs, Wilcoxon Signed-Ranks tests, Kruskal-Wallis ANOVAs, and chi-squares. Morris water maze data were analyzed with MANOVAs. HPA axis hormone data were analyzed using MANOVAs (corticosterone and ACTH) and ANOVAs (CRF).

Values of eta squared were used to determine the relative magnitude of stress effects for subgroups. Eta squared is a measure of effect size that indicates the proportion of variance explained by a given independent variable.

In analysis of variance terms, it is the ratio of the between-groups sum of squares to the total sum of squares (Cohen & Cohen, 1983).

All tests were two-tailed with  $p < 0.05$ . Several strategies were employed to minimize the probability of Type I error. First, the experiment was designed to provide adequate power (i.e., 0.80). When sample size supports adequate power, the likelihood of Type I errors is minimized. Second, global analyses incorporating all factors (Strain, Sex, Stress) were used to guide internal analyses. That is, analyses of subgroups were pursued only if overall analyses reveal significant main effects or interactions. This strict Fisherian strategy is consistent with recommendations of Keppel (1991) and Cohen and Cohen (1983), and substantially reduces the number of tests performed. Third, conservative analytic approaches were used wherever appropriate (i.e., MANOVAs rather than ANOVAs). Fourth, the error term (the within-subjects variance that constitutes the denominator of the F ratio) specific to the comparison being made was used rather than the error term from all subjects. This technique controls Type I error because as the denominator degrees of freedom decrease, the F value necessary to achieve significance for a given comparison increases.

## RESULTS

### **Body weight.**

Body weight is the outcome of energy intake and energy expenditure (including activity levels as well as metabolic factors). This dependent measure is useful in rodents because it reflects general health status (i.e., growth appropriate to age) and is sensitive to stress. Previous work (Zylan & Brown, 1996; Faraday et al., 1998) has suggested, however, that there may be differences among subgroups considered in this experiment in the extent to which stress affects body weight. These subgroups — Sprague-Dawley males, Sprague-Dawley females, Long-Evans males, Long-Evans females — are conceptualized as representing subpopulations of normal subjects that differ in vulnerability to stress effects on behavior and, ultimately, health.

**Analytic approach. Baseline Phase.** An ANOVA was run on body weights from the last Baseline day to verify that animals assigned to stress and no-stress groups were statistically indistinguishable.

**Stress Phase.** Body weight data from the Stress phase were analyzed in two ways to obtain different types of information. Repeated-measures analyses were used to test for within-subject effects (i.e., effect of Time), interactions of within-subject effects with between-subjects factors (e.g., Time X Strain, Time X Sex, Time X Stress), and effects of between-subjects factors averaged over Time. These analyses revealed the extent to which the independent variables altered growth rates of subjects and the extent to which the independent

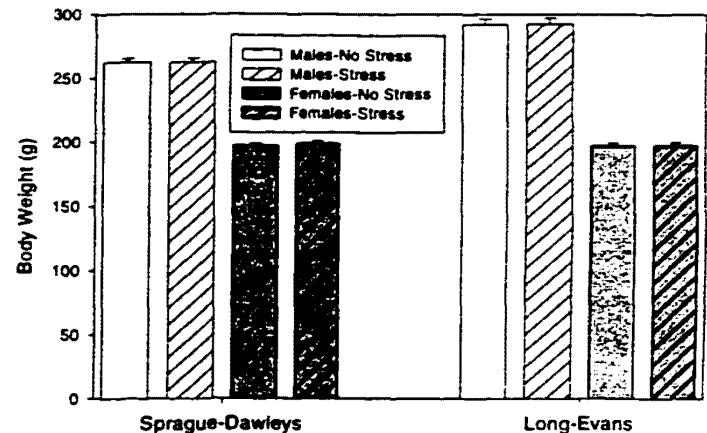
variables altered body weight collapsed across Time.

Two sets of repeated-measures analyses were run: one set on data obtained from Stress Day 1 through Stress Day 11 and a second set on data obtained from Stress Day 1 through Stress Day 21. This approach was used because on Stress Day 11 four animals from each treatment cell were sacrificed. Analyses through Stress Day 11, therefore, included all animals (N=167) and reveal the effects of daily stress for about one and a half weeks. Analyses through Stress Day 21 included animals that finished the entire experiment (n=135), and indicate the effects of three weeks of daily stress.

Then, ANOVAs were used to evaluate between-groups differences on each measurement day. These analyses provided a finer-grained evaluation of between-groups differences by indicating the extent to which independent variables altered body weight on each measurement day.

For each type of analysis, all animals first were considered together. Because sex differences in body weight were the largest differentiating variable, the sexes then were analyzed separately. Analysis results at this level determined whether the subgroups (Sprague-Dawley males, Sprague-Dawley females, Long-Evans males, Long-Evans females) also were examined separately. F values, degrees of freedom, and p values for each test are reported in Tables 20 - 23 in Appendix A. All effects and tests reported are significant at  $p < 0.05$  unless otherwise noted.

**Baseline analyses.** See Figure 8 and Table 17 (Appendix A). When all animals were considered, Long-Evans animals weighed more than Sprague-Dawleys, males weighed more than females, and Strain interacted with Sex such that Long-Evans males were heavier than Sprague-Dawley males but female animals weighed similar amounts. The greater body weights of Long-Evans males than Sprague-Dawley males of the same age is consistent with growth curves reported by the breeder (Charles River Laboratories). There were no differences between animals assigned to no stress and stress groups when subgroups were examined separately.



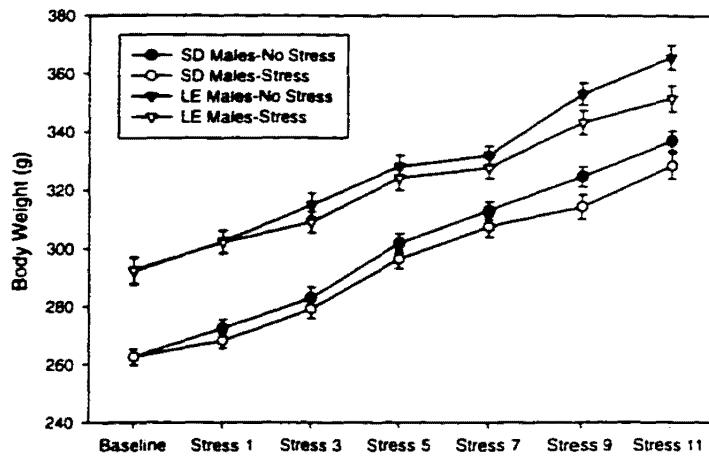
**Figure 8: Body weights of treatment groups on last day of Baseline period.**

**Stress Phase analyses: *Repeated-measures analyses through Stress Day 11.*** See Figures 9 and 10, Table 2 below, and Tables 18 and 19 (Appendix A). These analyses reveal effects of one and a half weeks of daily stress. Animal body weights increased over Time [ $F(5, 795)=1117.37$ ] as expected during the dynamic growth phase. Long-Evans animals grew larger than did Sprague-Dawleys, males had greater increases than did females, and Stress reduced body weight gains [Time X Stress:  $F(5, 765)=9.49$ ]. A Time X Strain X Sex interaction indicated that Long-Evans males were always heavier

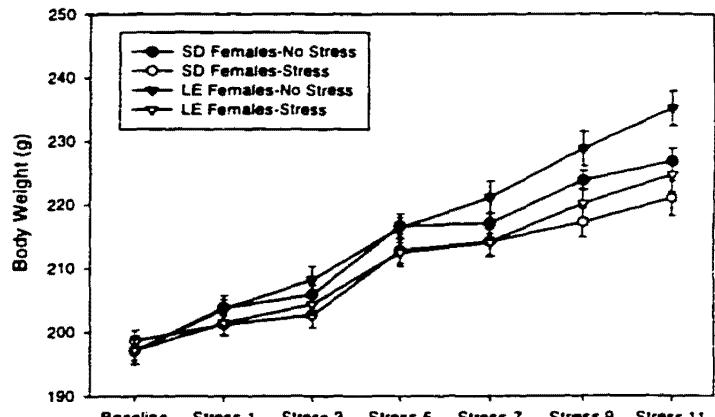
than Sprague-Dawley males while females began the experiment at similar weights but Long-Evans females became heavier than Sprague-Dawley females over time. These Strain, Sex, Stress, and Strain X Sex differences also were evident when body weights were averaged over Time.

When the sexes were considered separately, animals gained weight over Time, Long-Evans animals gained more weight than did Sprague-Dawleys, and Stress reduced body weight gains for males [ $F(5, 390) = 5.50$ ] and for females [ $F(5, 405) = 4.04$ ]. When body weights were averaged over time, Stress reduced body weights of males and females. Strain differences were present when body weights were averaged only for males.

When the subgroups were considered separately, growth over time was evident in all groups. Stress reduced body weight gains only for Long-Evans males [Time X Stress:  $F(5, 190) = 5.75$ ] and Long-Evans females [Time X Stress:  $F(5, 190) = 3.00$ ]. When body weights were averaged over time, Stress effects were present only.



**Figure 9: Body weights of males through Stress Day 11.**



**Figure 10: Body weights of females through Stress Day 11.**

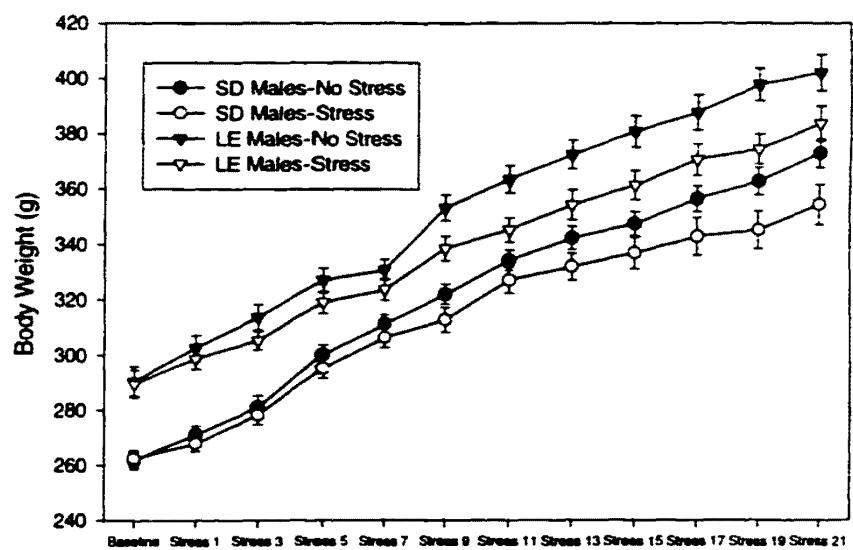
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among Long-Evans females. One and a half weeks of daily stress did not reduce rates of body weight gain for Sprague-Dawley males and females or reduce body weights on average over this period. Overall, therefore, Long-Evans males and females were most vulnerable to stress during this period.

**Table 2. Averaged body weights (g) from Stress Day 1 through 11.**

Group	n	Mean	sem	Group	n	Mean	sem
SD Males-No Stress	21	305.12	3.07	LE Males-No Stress	20	332.52	3.57
SD Males-Stress	21	298.80	3.08	LE Males-Stress	20	326.07	3.78
SD Females-No Stress	21	215.70	1.31	LE Females-No Stress	20	218.91	2.20
SD Females-Stress	24	211.54	1.98	LE Females-Stress	20	212.90	1.71

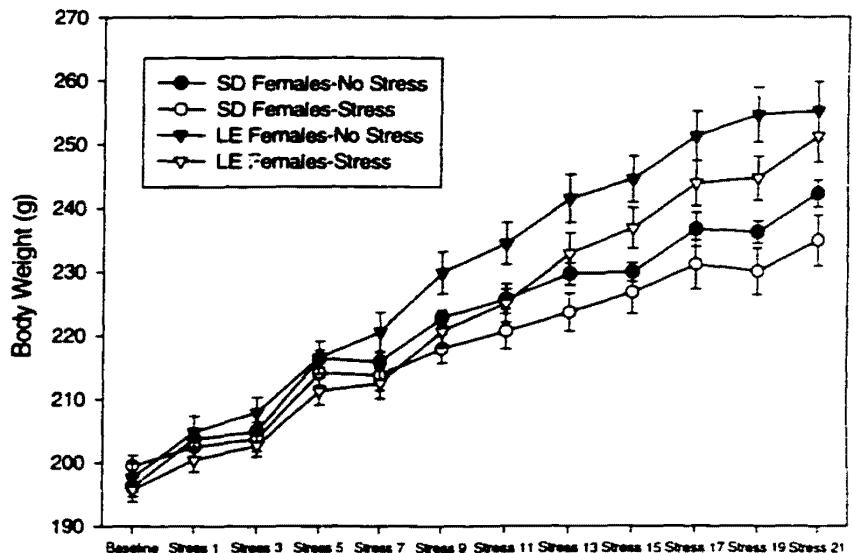
**Repeated-measures analyses through Stress Day 21.** See Figures 11 and 12, Table 3 below, and Tables 20 and 21 (Appendix A). Animal body weights increased over Time, Long-Evans were heavier than Sprague-Dawleys, males were heavier than females, and Stress reduced body weight gains [Time X Stress:  $F(10, 1270) = 7.40$ ]. Long-Evans males also were always heavier than Sprague-Dawley males, while Long-Evans females became heavier than Sprague-Dawley females partway through the experiment



**Figure 11: Body weights of males through Stress Day 21.**

(Time X Strain X Sex). In contrast to analysis results through Stress Day 11, the stress-induced reduction in body weight gain was greater for males than for females [Time X Sex X Stress:  $F(10, 1270)=2.72$ ]. Strain, Sex, Stress, and Strain X Sex differences also were evident when body weights were averaged over Time.

When the sexes were considered separately, growth over Time and greater growth by Long-Evans than by Sprague-Dawleys was evident among males and females. In contrast to effects of Stress through Stress Day 11, Stress reduced body weights of males over time [Time X Stress:  $F(10, 620)=6.36$ ] but did not reduce female body weight. Strain and Stress differences were evident on average for each sex.



**Figure 12: Body weights of females through Stress Day 21.**

The sex difference in Stress effects over Time also was evident when the subgroups were considered separately. Growth over Time was evident for each group and Stress reduced body weight gains for Sprague-Dawley males [ $F(10, 320)=3.09$ ] and Long-Evans males [ $F(10, 300)=3.83$ ] but not for females of either strain. On average, Stress reduced body weights of Long-Evans males ( $p<0.05$ ) and tended to reduce Long-Evans female body weight ( $p=0.08$ ). Over this longer

stress period, therefore, vulnerability to stress effects on body weight continued for Long-Evans males and females and emerged in Sprague-Dawley males. Sprague-Dawley female body weight, however, remained unperturbed by stress.

**Table 3. Averaged body weights (g) from Stress Day 1 through 21.**

Group	n	Mean	sem	Group	n	Mean	sem
SD Males-No Stress	17	327.38	3.76	LE Males-No Stress	16	357.34	4.70
SD Males-Stress	17	318.03	4.47	LE Males-Stress	16	343.11	4.31
SD Females-No Stress	17	223.99	1.14	LE Females-No Stress	16	232.85	3.10
SD Females-Stress	20	219.91	2.58	LE Females-Stress	16	225.63	2.55

***ANOVAs on each measurement day.*** See Tables 4 and 5 below, and Tables 22, 23, and 24 (Appendix A). ANOVAs were done to evaluate between-subjects differences on each measurement day — a finer-grained analysis of between-groups differences than provided by analysis of body weights averaged across Time. When all animals were considered together, Long-Evans were heavier than Sprague-Dawleys, males were heavier than females, and Long-Evans males were heavier than Sprague-Dawley males with smaller or no differences between strains of females (Strain X Sex) on each measurement day. In addition, Stress reduced body weights on Stress Days 3 through 21.

When the sexes were considered separately, among males Long-Evans were always heavier than Sprague-Dawleys. Among females, this difference appeared on Stress Day 9, when the Long-Evans female growth rate overtook the Sprague-Dawley female growth rate, and remained throughout the rest of the experiment. Effects of Stress to reduce body weight appeared early in females

and were present throughout the experiment until Stress Day 21. In contrast, for males effects of Stress to reduce body weight did not appear until Stress Day 9 and then persisted for the rest of the experiment (see Table 4 below).

**Table 4. Statistically significant ( $p < 0.05$ ) stress effects (yes/no) to reduce body weight within each sex on each Stress Day.**

Stress Day	1	3	5	7	9	11	13	15	17	19	21
<b>Males</b>	NO	NO	NO	NO	YES						
<b>Females</b>	NO	YES	NO								

When animals were further broken down into subgroups, it became clear that reductions in body weight as a result of Stress were strongest among Long-Evans animals (see Table 5 below).

**Table 5: Statistically significant ( $p < 0.05$ ) stress effects (yes/no) to reduce body weight within each subgroup on each Stress Day.**

Stress Day	1	3	5	7	9	11	13	15	17	19	21
<b>SD Males</b>	NO	NO	NO	NO	YES ( $p=0.06$ )	NO	NO	NO	NO	YES	YES
<b>SD Females</b>	NO	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO
<b>LE Males</b>	NO	NO	NO	NO	YES ( $p=0.09$ )	YES	YES	YES	YES	YES	YES
<b>LE Females</b>	NO	NO	NO	YES	YES	YES	YES	YES ( $p=0.09$ )	NO	NO	YES ( $p=0.08$ )

**Body weight summary.** Overall, Stress reduced rates of body weight gain as well as body weights on each measurement day. The magnitude of body weight reductions depended on the Sex and Strain of animal as well as on the length of Stress exposure. In particular, in response to one and half weeks of daily stress (analyses through Stress Day 11), growth rates of Long-Evans males and females but not of Sprague-Dawley males and females were reduced. The

reduction for Long-Evans females was robust enough to be evident when body weights were averaged across Stress Days 1 through 11. ANOVAs on each day confirmed these patterns, with Stress effects present for Sprague-Dawley males and females only on Stress Day 9, but present for Long-Evans males on Stress Days 9 and 11, and for Long-Evans females on Stress Days 7, 9, and 11.

In response to three weeks of daily stress, Stress also reduced rates of body weight gain and body weight measurements on each day with subjects' Strain and Sex affecting outcomes. In response to this longer stress period, Sprague-Dawley and Long-Evans males, but not females, exhibited significant reductions in body weight gains. Body weight reductions also were evident on average for Long-Evans males, but not for Sprague-Dawley males. In addition, on average, stress reduced body weights of Long-Evans females. ANOVAs on each day confirmed these patterns, with Stress effects evident for Sprague-Dawley males on Stress Days 9, 19, and 21, for Long-Evans males on Stress Days 9, 11, 13, 15, 17, 19, and 21, and for Long-Evans females on Stress Days 7, 9, 11, 13, and 19. Sprague-Dawley female body weight remained relatively unperturbed by Stress, with no Stress effects on growth rate, no average Stress effects collapsed across Time, and a significant stress effect on only one day (Stress Day 9) of the Stress phase.

Average eta-squared statistics were calculated for Stress effects on body weight from Stress Day 7 (the day on which Stress effects first appeared in a subgroup) through Stress Day 21 to assess the magnitude of effects for each subgroup. Average effect size was largest for Long-Evans males, with Stress

accounting for 13% of body weight variance. Female Long-Evans had the second largest effect size, with Stress accounting on average for 9.5% of body weight variance. For Sprague-Dawley males, Stress effects averaged 8% of body weight variance. Effects were smallest among Sprague-Dawley females, with Stress accounting nonsignificantly for 5.5% of variance on average.

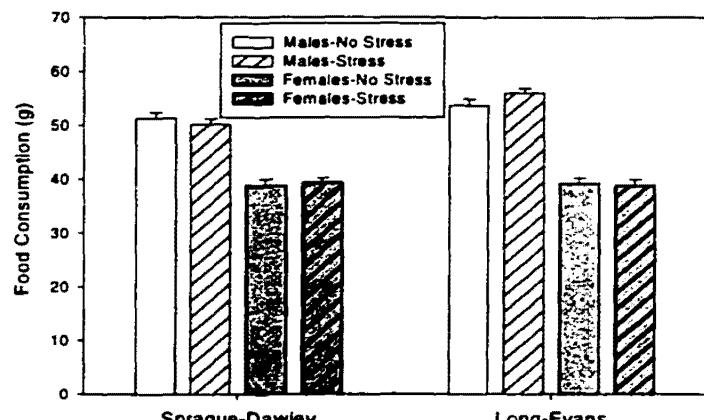
**Implications: Vulnerability vs. Resilience.** Long-Evans males were the most sensitive of the subgroups to stress-induced body weight reductions, followed by Long-Evans females as the next most sensitive and then by Sprague-Dawley males. Sprague-Dawley females were resistant to these stress effects — a pattern unique among the subgroups. This pattern of findings suggests that males, and especially Long-Evans males, may possess certain biologically-based attributes (i.e., a specific vulnerability) that is manifested by and correlated with stress-induced changes in body weight. The next question to be answered was whether reductions in body weight were simply the result of reduced feeding by the three affected subgroups.

## **Food consumption.**

Stress effects on body weight could be the result of changes in energy intake or changes in energy expenditure. This section addresses the question of whether body weight reductions in Long-Evans males and females and in Sprague-Dawley males were the consequence, at least in part, of reduced food consumption.

**Analytic approach.** The analytic approach to food consumption data was identical with the strategy used to evaluate body weight data. Briefly, repeated-measures analyses were used to examine within-subject effects, interactions of within-subject effects with between-subjects effects, and averaged between-subjects effects for all animals through Stress Day 11 (N=167) and for the majority subset of animals that completed the experiment through Stress Day 21 (n=135). ANOVAs were used to evaluate differences at Baseline between treatment groups and to assess between-subjects effects on each measurement day. F values, degrees of freedom, and p values for each test are reported in Tables 25 - 32 in Appendix A. All effects and tests reported are significant at  $p < 0.05$  unless otherwise noted. Units are grams of food consumed during each two-day period.

### **Baseline analyses. See**

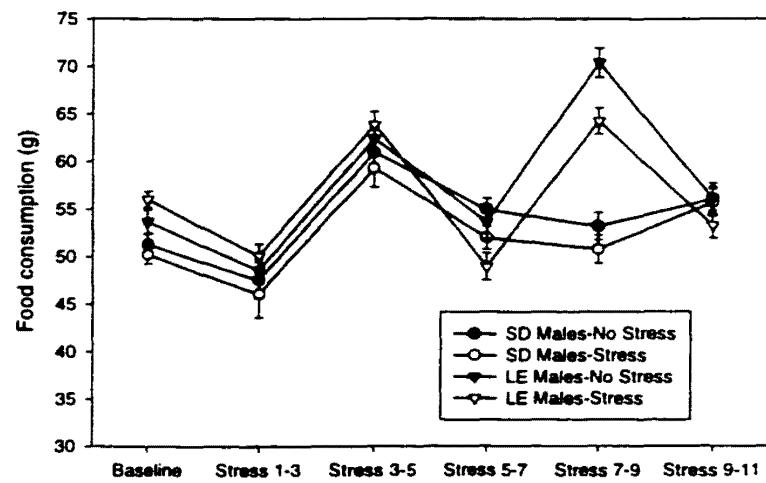


**Figure 13: Food consumption of all treatment groups on last two days of Baseline period.**

Figure 13 and Table 25 (Appendix A). When all animals were considered, Long-Evans animals ate more than did Sprague-Dawleys, males ate more than did females, and Long-Evans males ate more than Sprague-Dawley males while females of each strain ate similar amounts (Strain X Sex). These Sex and Strain differences parallel Sex and Strain differences in baseline body weight. Food consumption did not differ between animals assigned to no stress and stress groups when subgroups were examined separately.

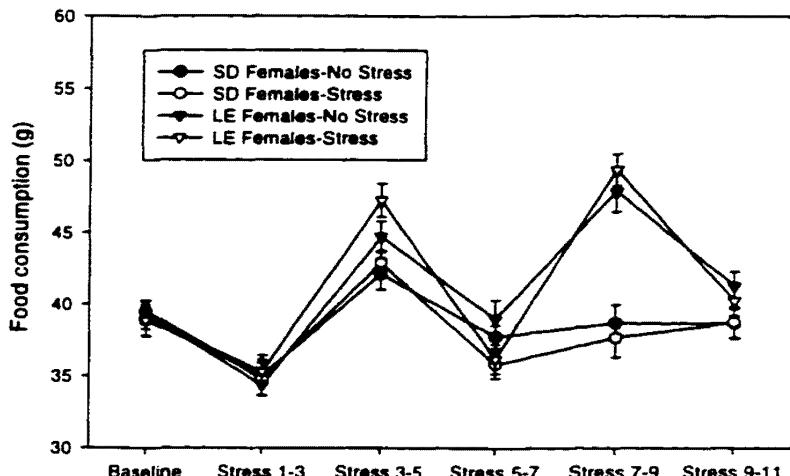
**Stress Phase analyses. Repeated-measures analyses through Stress Day 11.** See Figures 14 and 15, Table 6 below, and Tables 26 and 27 (Appendix A). These analyses reveal effects of one and a half weeks of daily stress. Food consumption changed over Time, Long-Evans generally ate more than did Sprague-Dawleys, males ate more than did females, and Stress reduced feeding [Time X Stress:  $F(4,636)=3.85$ ]. In addition, Long-Evans males ate more than did Sprague-Dawley males with smaller magnitude differences between females of each strain

(Time X Strain X Sex). When food consumption was averaged over Time, these Strain and Sex differences were evident ( $p<0.05$ ), and Stress effects also tended to be present ( $p=0.08$ ).



**Figure 14. Male food consumption for two-day periods through Stress Day 11.**

When the sexes were considered separately, feeding increased over Time and Long-Evans males and females ate more than did Sprague-Dawley males and females. In addition, Stress decreased feeding for males



**Figure 15. Female food consumption for two-day periods through Stress Day 11.**

[Time X Stress:  $F(4, 312)=2.52$ ] and for females [Time X Stress:  $F(4, 324)=2.19$ ,  $p = 0.07$ ]. These Strain ( $p<0.05$ ) and Stress differences ( $p=0.06$ ) also were clear among males when food consumption was averaged over Time. averaged In contrast, only the Strain difference was evident on average among females.

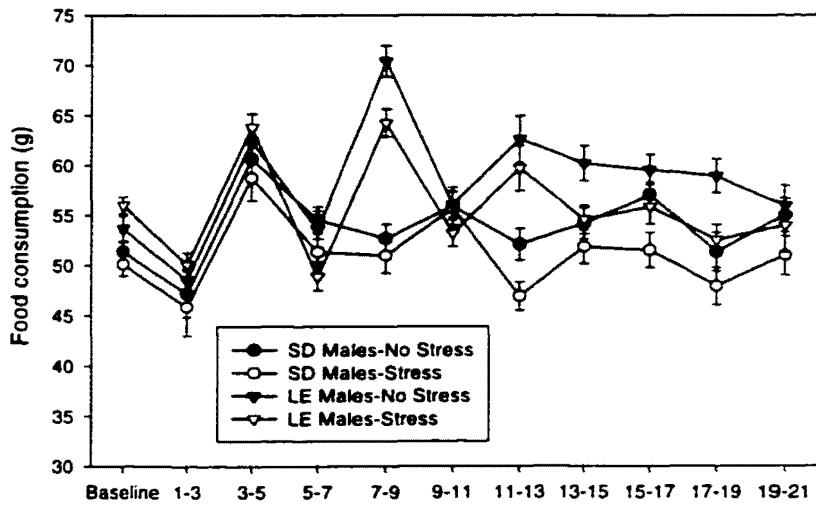
When the subgroups were considered separately, Stress reduced feeding for Long-Evans males [Time X Stress:  $F(4, 152)=5.60$ ] and altered Long-Evans female feeding [Time X Stress:  $F(4, 152)=2.94$ ] in a complex pattern, with increases on some days and decreases on other days. Sprague-Dawley feeding was not affected by Stress. Stress did not alter feeding for any subgroup when food consumption was averaged over Time.

**Table 6. Averaged two-day food consumption (g) from Stress Day 1 through 11.**

Group	n	Mean	sem	Group	n	Mean	sem
SD Males-No Stress	21	54.53	1.02	LE Males-No Stress	20	58.22	1.06
SD Males-Stress	21	52.75	1.05	LE Males-Stress	20	56.07	0.96
SD Females-No Stress	21	38.44	0.52	LE Females-No Stress	20	41.44	0.81
SD Females-Stress	24	37.97	0.62	LE Females-Stress	20	41.67	0.68

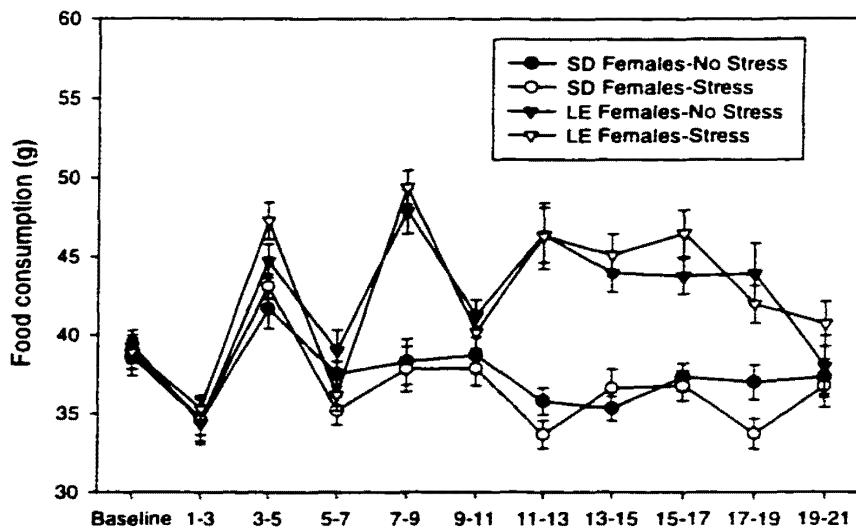
**Repeated-measures analyses through Stress Day 21.** See Figures 16 and 17, Table 7 below, and Tables 28 and 29 (Appendix A). These analyses reveal the consequences of three weeks of daily stress on food consumption. When all animals were considered together, feeding patterns changed over time and Long-Evans animals ate more than Sprague-Dawleys. Stress also generally reduced food consumption [Time X Stress:  $F(9,1143)=2.67$ ]. During this longer period, Long-Evans females consumed more food than did Sprague-Dawley females, with differences in the same direction but of smaller magnitude among the two strains of males (Time X Strain X Sex). When food consumption was averaged over Time, these Strain, Sex, and Stress differences also were evident. In addition, Stress decreased male feeding but not female feeding [Sex X Stress:  $F(1, 127)=4.85$ ].

When the sexes were examined separately, feeding increases over Time and Strain differences over time remained. In addition, Stress reduced feeding for males [Time X Stress:  $F(9, 558)=1.71$ ,  $p=0.08$ ] and altered feeding for females [Time X Stress:  $F(9, 585)=2.00$ ]. Similar to results through



**Figure 16.** Male food consumption over two-day periods through Stress Day 21.

Stress Day 11, when food consumption was averaged, Strain differences and Stress-induced feeding reductions were evident among males, but only Strain differences were apparent among females.



**Figure 17. Female food consumption over two-day periods through Stress Day 21.**

When the subgroups were examined separately, all groups altered feeding over time but only Long-Evans male feeding was reduced by Stress [Time X Stress:  $F(9,270)=2.60$ ]. When food consumption was averaged, Stress tended to reduce consumption by Sprague-Dawley males ( $p=0.09$ ) and Long-Evans males ( $p=0.077$ ) but not by females of either strain.

**Table 7. Averaged two-day food consumption (g) from Stress Day 1 through 21.**

Group	n	Mean	sem	Group	n	Mean	sem
SD Males-No Stress	17	54.08	1.05	LE Males-No Stress	16	58.75	1.28
SD Males-Stress	17	51.19	1.28	LE Males-Stress	16	55.42	1.30
SD Females-No Stress	17	37.36	0.38	LE Females-No Stress	16	42.13	0.97
SD Females-Stress	20	36.62	0.58	LE Females-Stress	16	42.82	0.79

**ANOVAs on each measurement day.** See Tables 8 and 9 below, and Tables 30, 31, and 32 (Appendix A). ANOVAs on each measurement day were performed to more closely examine daily between-groups differences. When all

animals were considered together, males ate more than did females during every measurement period. Long-Evans also generally ate more than did Sprague-Dawleys (during every measurement period except for Stress 1-3, 5-7 and 9-11). Stress reduced food consumption during measurement periods of Stress 5-7 [ $F(1, 159)=12.63$ ], Stress 7-9 [ $F(1, 159)=4.30$ ], Stress 11-13 [ $F(1, 127)=4.66$ ], Stress 15-17 [ $F(1, 127)=3.31$ ,  $p=0.07$ ], and Stress 17-19 [ $F(1, 127)=11.75$ ].

When the sexes were examined separately, greater food consumption by Long-Evans compared to Sprague-Dawleys also was evident during most measurement periods (except for Stress 1-3, 5-7, and 19-21, and Stress 9-11 for males). In contrast to these relatively stable strain differences within each sex, there were marked sex differences in effects of stress on feeding (see Table 8 below). Stress reduced feeding by males during six of the ten measurement periods while female feeding was reduced during only two periods.

Table 8: Statistically significant ( $p < 0.05$ ) stress effects (yes/no) to reduce feeding within each sex during each Stress measurement period.										
Stress Period	1-3	3-5	5-7	7-9	9-11	11-13	13-15	15-17	17-19	19-21
Males	NO	NO	YES	YES	NO	YES	YES	YES	YES	NO
Females	NO	NO	YES	NO	NO	NO	NO	NO	YES	NO

When the subgroups were examined separately (see Table 9 below), these sex differences remained, with Sprague-Dawley and Long-Evans male feeding most consistently reduced by Stress. Effects of Stress among Sprague-Dawley females were limited to feeding decreases during Stress 17-19 [ $F(1, 35)=5.05$ ]. There were no effects of Stress on feeding for Long-Evans females.

**Table 9: Statistically significant ( $p < 0.05$ ) stress effects (yes/no) to reduce feeding within each subgroup during each Stress measurement period.**

Stress Day	1-3	3-5	5-7	7-9	9-11	11-13	13-15	15-17	17-19	19-21
SD Males	NO	NO	YES ( $p=0.08$ )	NO	NO	YES	NO	YES	NO	NO
SD Females	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO
LE Males	NO	NO	YES	YES	NO	NO	YES	NO	YES	NO
LE Females	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO

**Food consumption summary.** Overall, Stress altered rates of food consumption and food consumption on each measurement day. The directionality and magnitude of feeding alterations depended on the Sex and Strain of animal. In particular, daily Stress for one and half weeks (analyses through Stress Day 11) reduced food consumption of males, and especially of Long-Evans males, but generally not of females. Effects among males were robust enough to be evident when food consumption was averaged across Stress Days 1 through 11. ANOVAs on each day confirmed these patterns, with Stress reducing feeding for Sprague-Dawley and Long-Evans males during this period but not for females of either strain.

In response to three weeks of daily stress, Stress also altered rates of food consumption and food consumption on each day with subjects' Strain and Sex affecting outcomes. Effects of Stress on food consumption during this longer period were similar to Stress effects through Day 11. For males, and especially for Long-Evans males, Stress reduced food consumption. These effects were sufficiently robust to be evident for all males as well as for Long-

Evans and Sprague-Dawley males separately when food consumption was averaged over the entire Stress phase. ANOVAs on each day confirmed these patterns, with Stress effects evident for males over most of the Stress phase but virtually absent for females of each strain.

Average eta-squared statistics were calculated for Stress effects on food consumption from measurement periods Stress 5-7 (the period during which Stress effects first appeared in a subgroup) through Stress Day 21 to assess the magnitude of effects for each subgroup. Average effect size was largest for Long-Evans males, with Stress accounting for 10.5% of food consumption variance. Sprague-Dawley males had the second largest effect size, with Stress accounting for 7.3% of feeding variance. Effect sizes among females were relatively small, with Stress explaining on average 3.6% of food consumption variance among Sprague-Dawleys and only 3.1% for female Long-Evans.

The marked sawtooth pattern present through Stress Day 11 in Figures 15-18 above warrants a comment. It may be relevant that the dips in feeding during Stress periods 1-3, 5-7, 9-11, and to a lesser extent 19-21 coincided with locomotor measurements — each animal was measured for two hours on Stress Days 1, 6, 9, and 19. Pilot work (see Figures 1 and 2) also revealed feeding decreases on Stress Day 4 and a smaller reduction on Day 10 — both days during which locomotion was measured. The fact that locomotion measurement may have decreased feeding, and compensatory increased feeding occurred in measurement periods immediately afterward, suggests that the locomotion experience was stressful despite the fact that animals had undergone two

acclimation exposures and one baseline exposure prior to measurements obtained during the Stress Phase. If the locomotion experience was stressful, then it also is worth noting that Sprague-Dawley feeding stabilized by Stress measurement period 5-7 while Long-Evans feeding was altered in response to every locomotion measurement throughout the experiment. These observations suggest that Sprague-Dawleys habituated to this “stressor” but Long-Evans did not. The methodologic and substantive issues raised by the potential impact of locomotion measurement on feeding, and possible strain differences in this effect, are addressed in the Discussion.

**Implications: Vulnerability vs. Resilience.** Overall, males were sensitive to stress-induced feeding reductions while females were resistant to these stress effects. Long-Evans males were the most sensitive of the male subgroups just as they were most sensitive to stress-induced body weight reductions. This pattern of findings suggests that males, and especially Long-Evans males, may possess certain biologically-based attributes (i.e., a specific vulnerability) that is manifested by and correlated with stress-induced reductions in body weight as well as in feeding. Importantly, stress-induced reductions in feeding paralleled stress-induced reductions in body weight for Sprague-Dawley and Long-Evans males. Long-Evans females, however, exhibited reduced body weights without a change in feeding, suggesting that changes in energy expenditure may be relevant for this subgroup. In addition, the fact that feeding changes did not parallel body weight changes for Long-Evans females suggests that this subgroup may carry a qualitatively different type of stress vulnerability.

## **Locomotion.**

Locomotion activity variables (i.e., horizontal activity and total distance) reflect overall activity levels and can be used as an index of physically-induced energy expenditure. Activity also reflects general health (i.e., animals that are ill or fearful exhibit reduced activity). Other locomotion variables — vertical activity and time spent in the center of the open field — were used as evidence for different aspects of Stress effects. In particular, vertical activity reflects exploration and center time may indicate fear- or anxiety-like states, with more time in the center indicating less anxiety.

In this experiment, locomotion was measured for 2 hours periodically, and therefore constitutes a partial activity index. Effects of Stress to reduce body weight of males across strains were paralleled by decreased food consumption but also might, in part, be accounted for by increases in activity. For Long-Evans females, Stress-induced decreases in body weight were not mirrored by decreased food consumption, suggesting that activity increases might be particularly important for this subgroup to account for body weight decreases.

**Analytic approach.** Locomotion data (horizontal activity, total distance, vertical activity, and center time) were analyzed using multivariate analyses of variance (MANOVAs) on each measurement day with ANOVAs to determine which individual variables contributed to multivariate differences. MANOVAs evaluate between-groups differences by allowing for the intercorrelation among related dependent variables and selecting the linear combination of variables

that best distinguishes groups. This linear combination is different for each independent variable (just as ANOVAs with more than one independent variable collapse across the other independent variables to assess for each main effect) and yields a multivariate F test for each independent variable. Because MANOVAs use information (i.e., correlations among variables and linear combinations of variables) that ANOVAs (univariate F-tests) do not use, it is possible to have a significant multivariate F without some or all of the univariate F-tests being significant and vice versa. A significant multivariate F in the absence of univariate significance indicates that small differences across the variables distinguish the groups even though the size of the difference on any single variable is insufficient to stand alone as statistically significant. The absence of multivariate significance with one or more significant univariate tests indicates the opposite situation in which a robust difference on one or a few variables is insufficient to distinguish the groups when all of the variables are taken into account (Stevens, 1996).

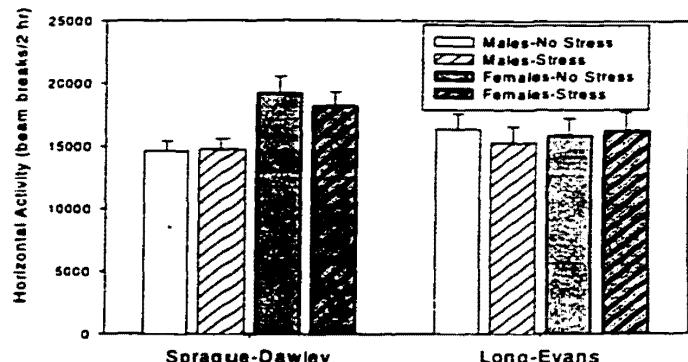
F values, degrees of freedom, and p values for each test are reported in Tables 33 - 38 in Appendix A. All effects and tests reported are significant at  $p < 0.05$  unless otherwise noted. Multivariate effects are indicated with an underscored capital F (i.e.,  $\underline{F}$ ) and have four degrees of freedom in the F ratio numerator. Univariate tests are indicated with a capital F and have one degree of freedom in the F ratio numerator.

All animals first were analyzed together. Because sex differences in locomotor activity were the largest differentiating variable, the sexes then were

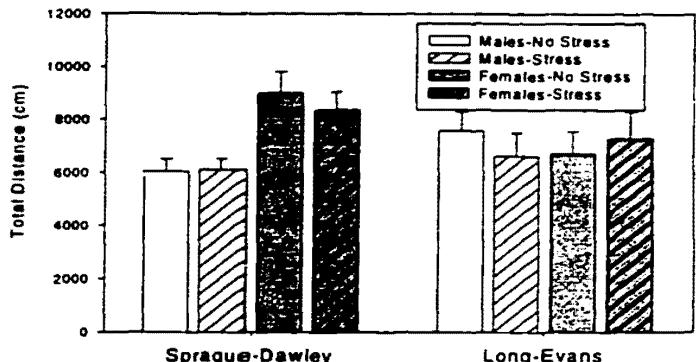
analyzed separately. Analysis results at this level determined whether or not subgroups also were examined separately.

**Baseline analyses.** See

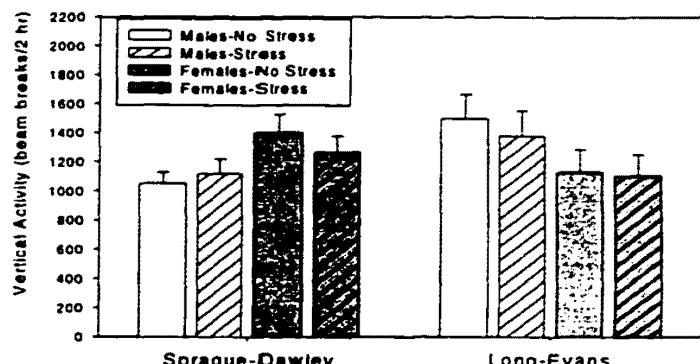
Figures 18-21 and Table 33 (Appendix A). When all animals were considered together, Long-Evans activity patterns differed from Sprague-Dawley patterns, females generally were more active than males, and these sex differences were more pronounced among Sprague-Dawleys than among Long-Evans (Strain X Sex). When the variables were considered separately, Strain differences were evident only in center time, with Sprague-Dawleys spending more time in the center than Long-Evans. Females exhibited more horizontal activity and total distance than males. Sprague-Dawley females were more active and covered more distance than did Sprague-Dawley males while



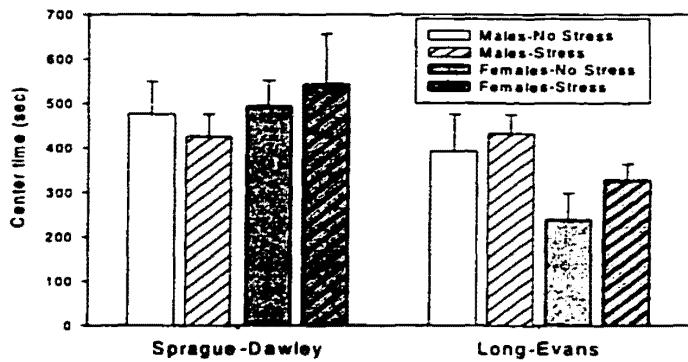
**Figure 18. Baseline horizontal activity of treatment groups.**



**Figure 19. Baseline total distance of treatment groups.**



**Figure 20. Baseline vertical activity of treatment groups.**



**Figure 21. Baseline center time of treatment groups.**

Long-Evans males and females were similarly active and moved similar distances (Strain X Sex).

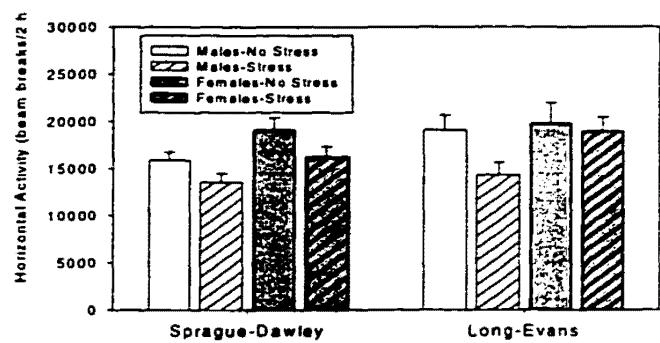
Sprague-Dawley females also exhibited greater vertical movement than did Sprague-Dawley males while Long-Evans males exhibited greater vertical movement than did Long-Evans females (Strain X Sex). Sprague-Dawley males and females spent similar amounts of time in the center, but Long-Evans males spent more time in the center than did Long-Evans females (Strain X Sex).

When subgroups were examined separately, there were no statistically significant differences on any variable between animals assigned to no stress and stress conditions.

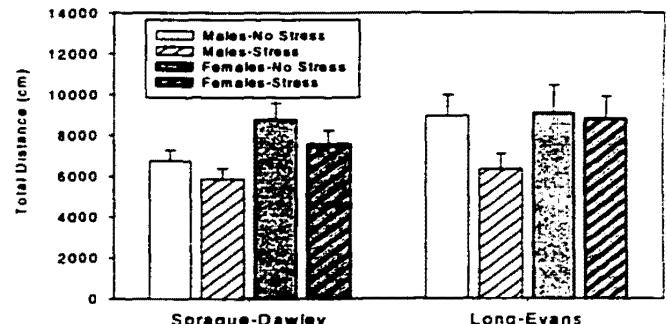
#### **Stress Phase analyses. Stress**

**Day 1.** See Figures 22-25, and Table 34 (Appendix A). When all animals were considered together, Long-Evans and Sprague-Dawley activity patterns differed, females were generally more active than males, and Stress generally reduced activity [ $F(4, 156)=2.96$ ].

Univariate tests indicated that Long-Evans animals exhibited greater horizontal and vertical activity, moved greater total distance, and spent more



**Figure 22. Horizontal activity on Stress Day 1.**



**Figure 23. Total distance on Stress Day 1.**

time in the center of the field than did Sprague-Dawleys. Females also exhibited more horizontal activity and moved greater distances than did males but spent less time in the center than did males. Stress reduced horizontal activity [ $F(1, 159)=7.60$ ], total distance [ $F(1, 159)=4.09$ ], and vertical activity [ $F(1, 159)=3.88$ ].

When the sexes were considered separately, Stress altered activity patterns [ $F(4, 75)=3.96$ ] among males but not among females. Among males, Stress reduced horizontal activity [ $F(1, 78)=8.81$ ], total distance [ $F(1, 78)=5.71$ ], and vertical activity [ $F(1, 78)=3.40$ ]. In addition, Long-Evans males exhibited greater total distance and vertical activity than did Sprague-Dawley males. When the male subgroups were examined separately, multivariate effects of Stress were present only among Long-Evans males [ $F(4, 35)=2.40, p=0.06$ ] for which Stress decreased horizontal activity [ $F(1, 38)=5.36$ ] and total distance [ $F(1, 38)=4.20$ ]. Among Sprague-Dawley males, Stress reduced horizontal

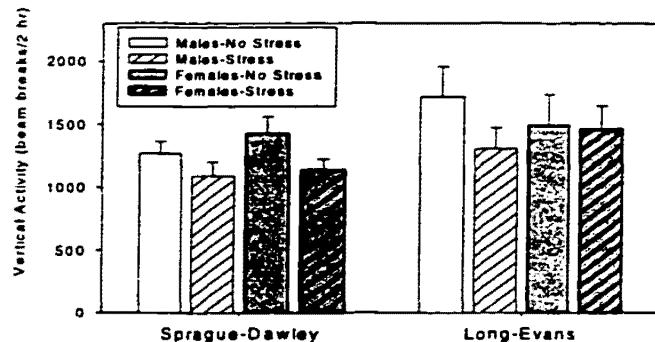


Figure 24. Vertical activity on Stress Day

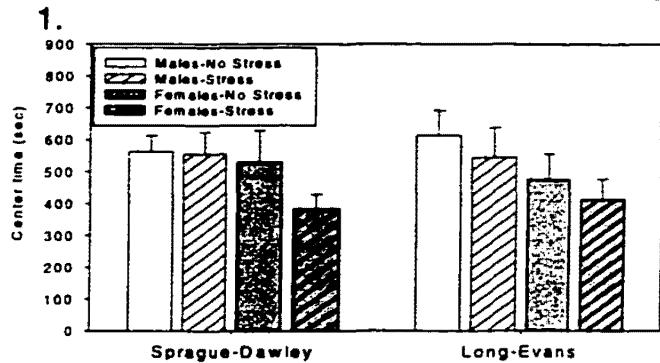


Figure 25. Center time on Stress Day 1.

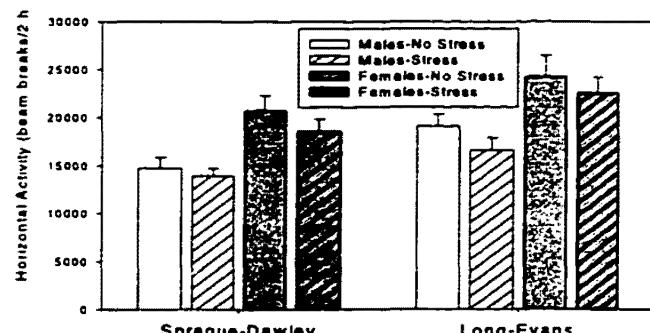


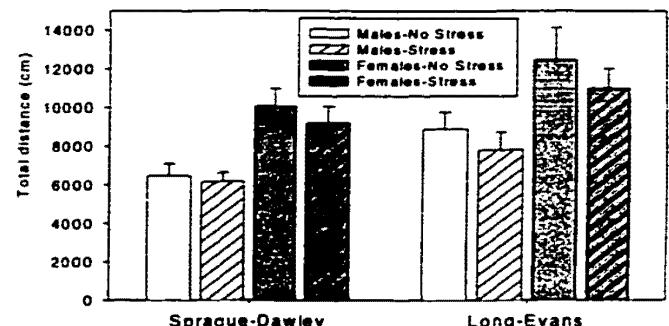
Figure 26. Horizontal activity on Stress Day 6.

activity [ $F(1, 40)=3.39, p=0.07$ ]. Female subgroups were not examined because of the lack of Stress effects.

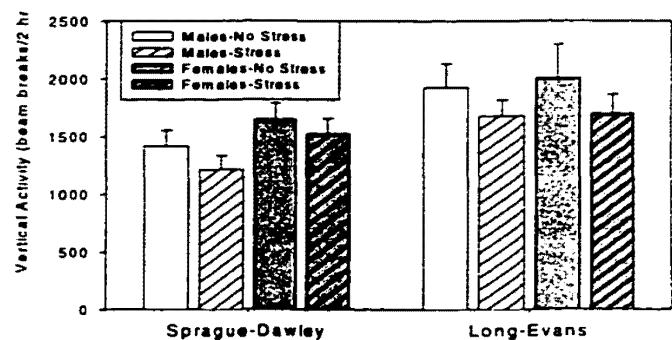
**Stress Day 6.** See Figures 26-29, and Table 35 (Appendix A). When all animals were considered together, Long-Evans and Sprague-Dawleys exhibited different activity patterns and females were generally more active than males.

Univariate tests indicated that Long-Evans exhibited greater horizontal activity, moved greater total distances, and exhibited more vertical activity than did Sprague-Dawleys. Females exhibited greater horizontal activity and moved greater distances than did males. Stress somewhat reduced horizontal activity [ $F(1, 159)=3.11, p = 0.08$ ] and vertical activity [ $F(1, 159)=3.26, p=0.07$ ].

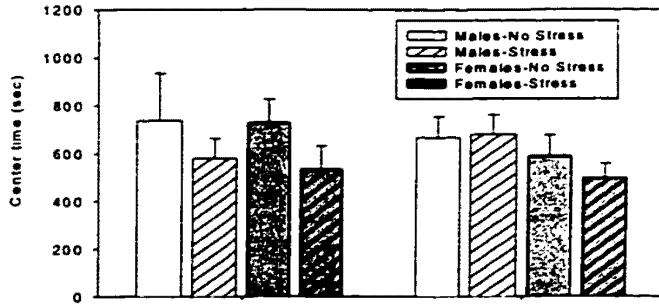
When the sexes were considered separately, multivariate Strain differences were present among males as well as females. Long-Evans males exhibited greater horizontal activity, moved greater distances, and exhibited greater vertical activity than did Sprague-Dawley males.



**Figure 27.** Total distance on Stress Day 6.



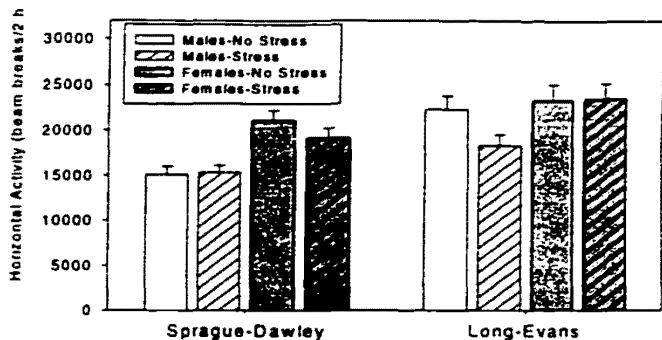
**Figure 28.** Vertical activity on Stress Day 6.



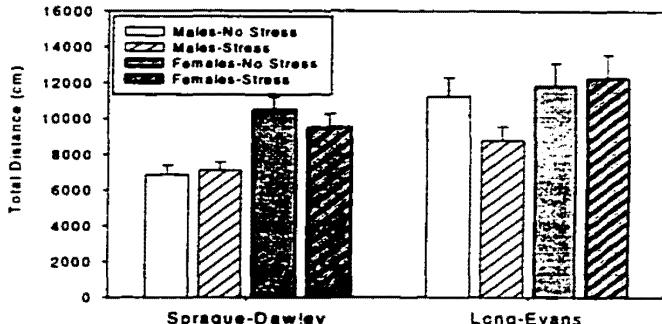
**Figure 29.** Center time on Stress Day 6.

Among females, Long-Evans exhibited greater horizontal activity and moved greater distances than did Sprague-Dawleys. In the absence of Stress effects, subgroups were not examined.

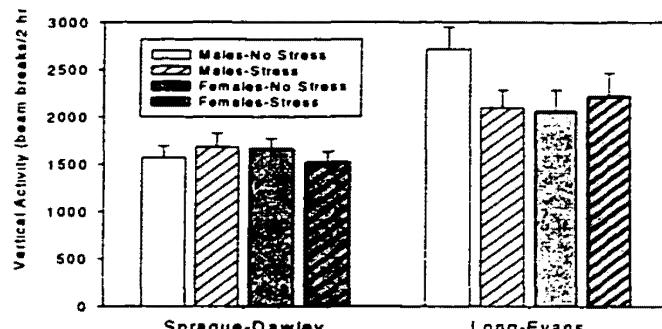
**Stress Day 9.** See Figures 30-33, and Table 36 (Appendix A). When all animals were considered together, Long-Evans and Sprague-Dawleys exhibited different activity patterns and females generally were more active than males. Long-Evans animals exhibited greater horizontal activity, moved greater distances, and exhibited greater vertical activity than did Sprague-Dawleys. Females also exhibited greater horizontal activity and moved greater distances than did males but spent less time in the center of the field than did males. Long-Evans males spent more time in the center than did Long-Evans females while Sprague-Dawley males and females had similar amounts of center



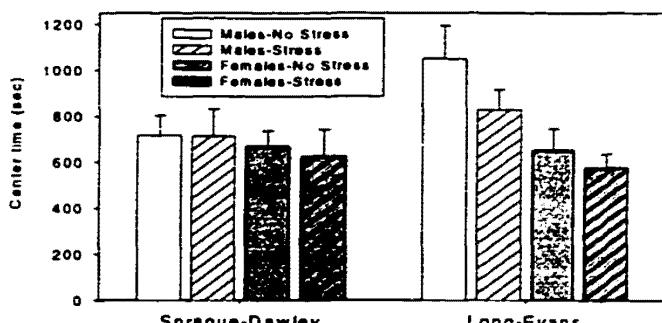
**Figure 30. Horizontal activity on Stress Day 9.**



**Figure 31. Total distance on Stress Day 9.**



**Figure 32. Vertical activity on Stress Day 9.**



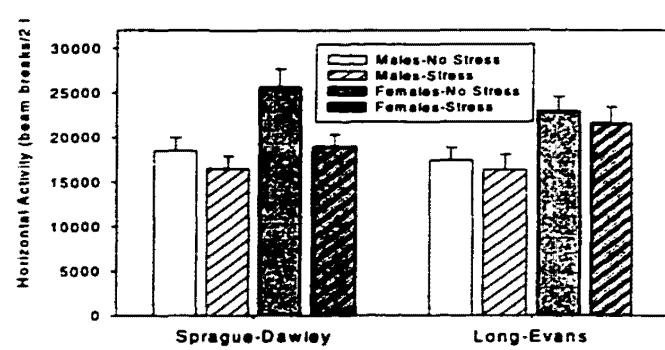
**Figure 33. Center time on Stress Day 9.**

time (Sex X Strain).

When the sexes were considered separately, Strain differences in activity patterns were present among males and among females. Among males, Long-Evans exhibited greater horizontal and vertical activity, moved greater distances, and spent more time in the center than did Sprague-Dawleys. In addition, Strain interacted with Stress such that stress reduced horizontal activity [ $F(1, 78)=3.55$ ], total distance [ $F(1, 78)=3.49$ ], and vertical activity [ $F(1, 78)=4.25$ ] of Long-Evans males but did not affect these behaviors of Sprague-Dawley males. Among females, Long-Evans exhibited greater horizontal and vertical activity, and moved greater distances than did Sprague-Dawleys.

Only male subgroups were considered separately because of the absence of Stress effects in females. There were no multivariate Stress effects for Long-Evans or Sprague-Dawley males. Among Long-Evans males, however, Stress reduced horizontal activity [ $F(1, 38)=4.41$ ], total distance [ $F(1, 38)=3.60$ ,  $p=0.066$ ], and vertical activity [ $F(1, 38)=4.20$ ]. Stress did not alter behaviors of Sprague-Dawley males.

**Stress Day 19.** See Figures 34-37, and Table 37 (Appendix A). When all animals were considered together, females generally were more active than males, exhibiting greater horizontal activity and moving more distance than males. Univariate tests also revealed that

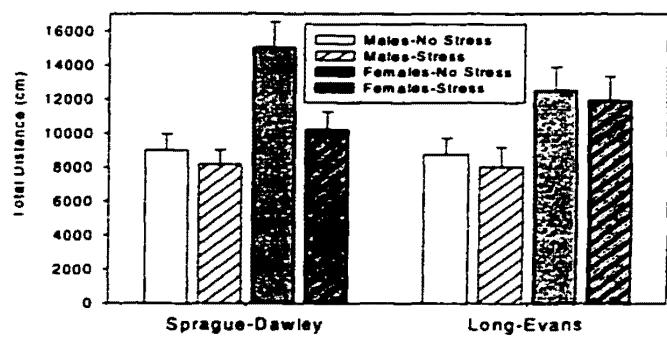


**Figure 34.** Horizontal activity on Stress Day 19.

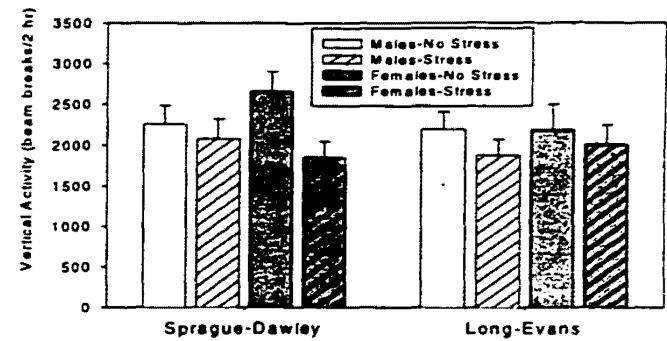
Sprague-Dawleys spent more time in the center of the field than did Long-Evans. Further, Stress reduced horizontal activity [ $F(1, 127)=5.92$ ], total distance [ $F(1, 127)=4.48$ ], and vertical activity [ $F(1, 127)=4.99$ ].

When the sexes were considered separately, multivariate tests were not significant but univariate tests among females revealed that Sprague-Dawley females spent more time in the center of the field than did Long-Evans females. In addition, Stress affected every variable among females, reducing horizontal activity [ $F(1, 65)=5.50$ ], total distance [ $F(1, 65)=4.18$ ], vertical activity [ $F(1, 65)=4.00$ ], and center time [ $F(1, 65)=3.34, p=0.07$ ].

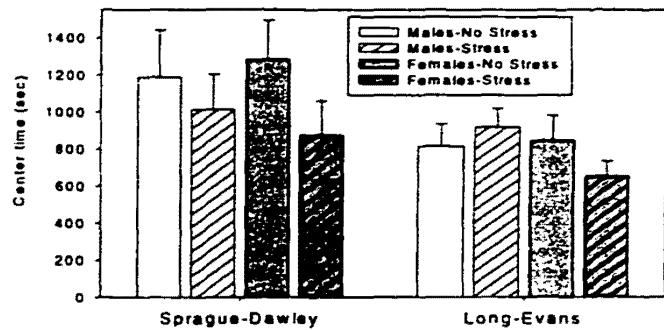
Only female subgroups were examined separately because of the absence of Stress effects among males. Stress tended to reduce activity [ $F(4, 32)=2.24, p=0.08$ ] among Sprague-Dawley females, reducing horizontal activity [ $F(1, 35)=8.05$ ], total distance [ $F(1, 35)=7.20$ ], and vertical activity [ $F(1,$



**Figure 35.** Total distance on Stress Day 19.



**Figure 36.** Vertical activity on Stress Day 19.



**Figure 37.** Center time on Stress Day 19.

35)=6.83].

**Locomotion summary. Effects of Stress on locomotion variables** depended on the Sex and Strain of animal and on the day of measurement. On Stress Day 1, Stress reduced activity variables (horizontal activity and/or total distance) of Long-Evans and Sprague-Dawley males. Effect size (eta squared) was greatest for Long-Evans males, with Stress accounting for about 11% of activity variance. The effect size for Sprague-Dawley males was half this size (5.5% of activity variance).

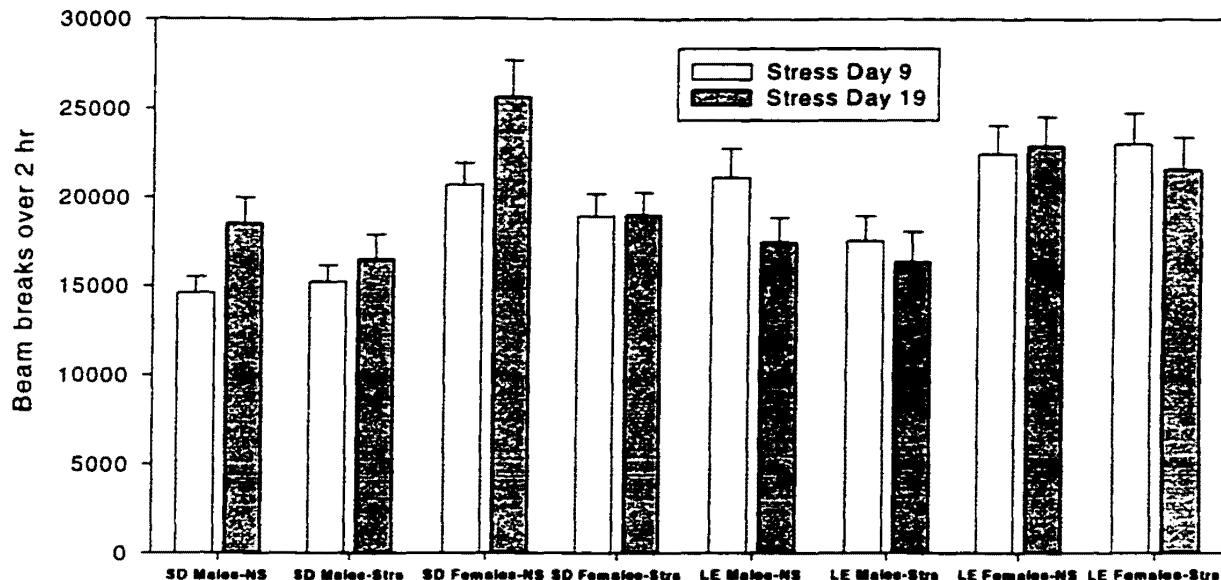
On Stress Day 9, Stress again reduced activity variables and exploratory behaviors of Long-Evans males, accounting for 9.5% of variance in activity and 10% of exploration variance. Over the first nine days of the Stress Phase, therefore, Long-Evans males were most consistently affected by stress. Sprague-Dawley and Long-Evans females were the least affected, with no significant multivariate or univariate Stress effects over the three measurement days.

On Stress Day 19, this pattern changed. Stress reduced activity, exploration, and time spent in the center of the open field for Sprague-Dawley females, accounting for 18% of activity variance, 16.3% of exploration variance, and 6% of center time variance, without affecting other subgroups. The emergence on Stress Day 19 of Stress effects in Sprague-Dawley females is striking, given that Stress had not altered this subgroup's locomotion before. It may be relevant that animals had not been in the locomotion chambers for the preceding ten days. Therefore, on this day subgroups of animals may have

experienced the locomotion chamber as novel and increased activity, exploration, and center time accordingly.

To determine whether activity patterns on Stress day 19 were significantly different from patterns on Stress Day 9, paired t-tests were used to compare activity, exploration, and center time for each subgroup across the two time points (See Figure 38 below and Table 38 in Appendix A). Interestingly, non-stressed Sprague-Dawley males and females significantly increased horizontal activity, total distance, vertical activity, and center time from Stress Day 9 to Stress Day 19, with greater increases by females. Stressed Sprague-Dawley males and females increased only vertical activity from Day 9 to Day 19. Non-stressed Long-Evans male horizontal activity decreased from Day 9 to Day 19. Locomotion behaviors of Long-Evans females did not change regardless of stress status.

These results suggest that Sprague-Dawley animals did experience the locomotion chamber as novel on Day 19 (indicated by increased activity and exploration among non-stressed animals) and that Stress suppressed activity increases but not exploration increases associated with novelty. The Stress effects in Sprague-Dawley females, then, were the result of the difference between the increased activity, exploration, and center time exhibited by non-stressed animals in the presence of novelty and the lack of these increases or smaller increases by stressed animals. The lack of changes in Long-Evans locomotion suggests that these subjects did not experience the chamber as novel on Day 19.



**Figure 38. Horizontal activity of each subgroup on Stress Day 9 and 19.**

Overall, these results reveal that changes in locomotion associated with Stress cannot account for Stress-associated decreases in body weight in males across strains and in Long-Evans females. In particular, Stress decreased activity in males — an effect opposite to that expected if activity changes were a source of body weight decrements. Metabolic factors (i.e., possibly via sex and stress hormones), therefore, must be important components of Stress-related body weight changes for males and, in particular, for Long-Evans females.

**Implications: Vulnerability vs. Resilience.** With regard to body weight, feeding, and activity variables, then, males appear to be more Stress-sensitive than females, with Long-Evans males the most affected across the three variable types, just as they were the most affected by Stress-induced body weight and feeding reductions. The next question to be answered is whether Stress sensitivity, once identified, occurs across all domains or whether sensitivity

patterns vary depending on the domain measured. In particular, does the marked sensitivity of Long-Evans males to repeated daily stress (as revealed by effects on body weight, feeding, and locomotion) predict impairments in reflexive cognitive processing (acoustic startle reflex with and without pre-pulse inhibition), in simple memory (passive avoidance), and in complex search strategy and spatial memory (Morris water maze)? Or, does sensitivity shift across domains, depending on the subgroup? These questions are important in terms of the potential utility of these changes to identify specific underlying vulnerability subtypes. In particular, sensitivity to Stress effects on body weight, feeding, and activity may indicate the presence of one specific type of stress vulnerability while sensitivity to cognitive effects of stress may indicate the existence of a different type of stress vulnerability. The next three sections address these questions in the context of cognitive responses to Stress.

### **Acoustic startle reflex (ASR) with and without pre-pulse inhibition (PPI).**

The acoustic startle reflex is an index of reactivity to a sudden, unexpected acoustic stimulus. The reflex also is altered in response to psychological or emotional states. When coupled with a preceding, non-startling pre-pulse, startle is reduced. This resultant pre-pulse inhibition reflects non-volitional, pre-conscious information-processing, sensory-gating, or attention.

This section is the first to deal with a behavioral measure that can be conceptualized as reflecting cognitive responses to stress. This section, and the sections that follow on passive avoidance and water maze performance, represent, therefore, the cognitive domain of stress effects in the present experiment — a domain quite different from the body weight, feeding, and activity variables reported in preceding sections.

**Analytic approach.** ASR and % PPI data (i.e., startle to 120 and 110 dB stimuli and the percentage of pre-pulse inhibition to each stimulus when paired with a 68 or 82 dB pre-pulse) were analyzed using separate multivariate analyses of variance (MANOVAs) on each measurement day with ANOVAs to determine which individual variables contributed to multivariate differences. Percent PPI was calculated according to the formula: [(Startle amplitude to stimulus without pre-pulse minus startle amplitude to the same stimulus when paired with a pre-pulse)/Startle amplitude to stimulus without pre-pulse] X 100.

F values, degrees of freedom, and p values for each test are reported in Tables 39 - 48 in Appendix A. All effects and tests reported are significant at  $p <$

0.05 unless otherwise noted. Multivariate effects are indicated with an underscored capital F (i.e.,  $\underline{F}$ ) and have two (startle amplitudes) or four (% PPI) degrees of freedom in the F ratio numerator. Univariate tests are indicated with a capital F and have one degree of freedom in the F ratio numerator.

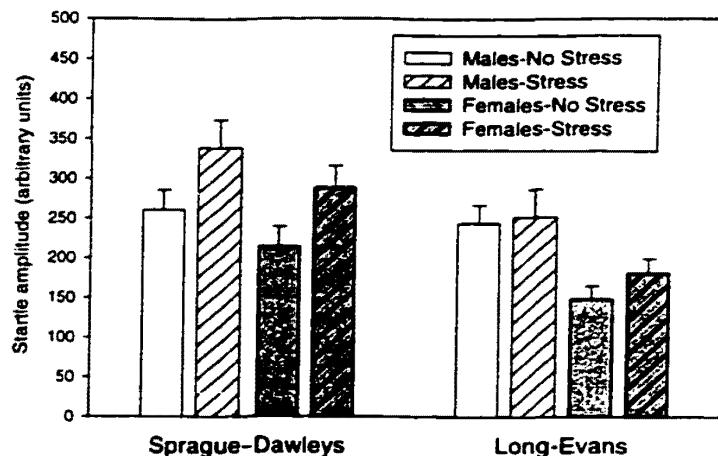
All animals first were analyzed together. Because strain differences in ASR and PPI were the largest differentiating variable, the strains then were analyzed separately. Analysis results at this level determined whether or not to examine subgroups separately. Results are reported and graphed for startle amplitude to the 120 dB stimulus and to the 110 dB stimulus, and for % PPI to each stimulus when preceded by an 82 dB pre-pulse. Results for the 68 dB pre-pulse are reported but were similar to those for the 82 dB pre-pulse and are not graphed separately.

**Baseline analyses.** See Tables 39 and 40 (Appendix A). MANOVAs were performed on baseline startle and % PPI values. Long-Evans females assigned to no stress and stress groups differed significantly in startle responses to the 120 dB stimulus. In addition, there were scattered differences in % PPI responses between animals assigned to no stress and stress groups. Therefore, analyses on responses during the Stress Phase were run with baseline responses as covariates.

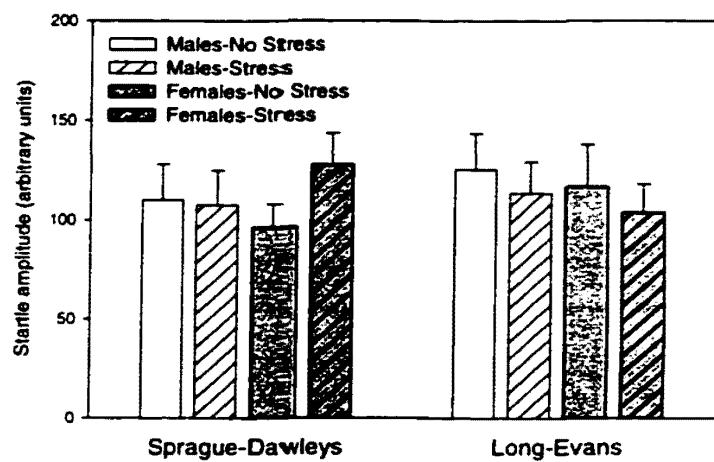
**Stress Phase Analyses. Stress Day 2 - Startle Amplitude.** See Figures 39 and 40, and Table 41 (Appendix A). When all animals were considered together, there were multivariate Strain, Sex, and Stress [ $\underline{F}(2,$

156)=6.38] differences. Univariate tests indicated that Long-Evans startled more to the 110 dB stimulus than Sprague-Dawleys, males startled more than females to the 120 dB stimulus, and Stress increased startle to the 120 dB stimulus [ $F(1, 157)=9.23$ ].

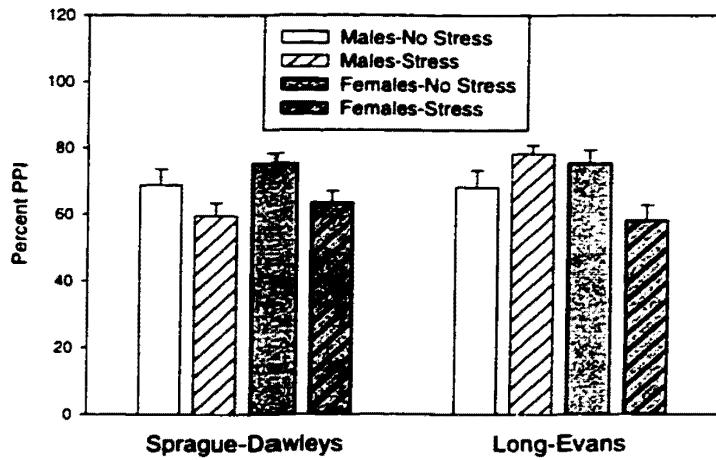
When the strains were considered separately, there were multivariate Sex ( $p=0.058$ ) and Stress differences [ $F(2, 80)=4.77$ ] among Sprague-Dawleys with males startling more than females and stressed animals startling more than non-stressed animals to the 120 dB stimulus [ $F(1, 81)=8.41$ ]. When Sprague-Dawley males and females were considered separately, there were multivariate Stress differences with Stress increasing startle to the 120 dB stimulus for males [ $F(1,$



**Figure 39.** Startle amplitude to 120 dB on Stress Day 2.

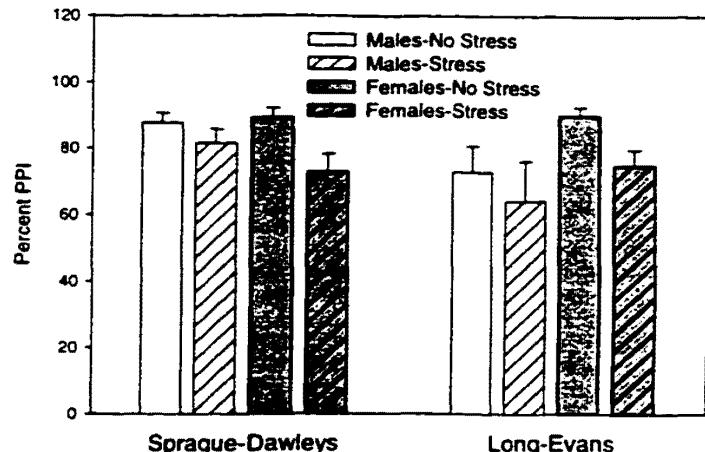


**Figure 40.** Startle amplitude to 110 dB stimulus on Stress Day 2.



**Figure 41.** Percent PPI to 120 dB with 82 dB pre-pulse on Stress Day 2.

38)=6.72] and for females [F(1, 41)=5.03]. Among Long-Evans, there was a multivariate trend for Sex differences ( $p=0.087$ ) but univariate tests were not significant. Because of the lack of Stress effects, no further tests were performed on Long-Evans subgroups.



**Figure 42.** Percent PPI to 110 dB stimulus with 82 dB pre-pulse on Stress Day 2.

**Stress Day 2 - % PPI.** See Figures 41 and 42, and Table 42 (Appendix A). When all animals were considered together, there were multivariate differences based on Stress status [F(4, 149)=3.69] and a Strain X Sex interaction. Stress decreased % PPI to the 120 dB stimulus [82 dB pre-pulse: F(1, 152)=4.87] and to the 110 dB stimulus regardless of pre-pulse dB level [68 dB: F(1, 152)=10.15; 82 dB: F(1, 152)=5.97]. Sprague-Dawley females had greater % PPI to the 120 dB stimulus with 82 dB pre-pulse than did Sprague-Dawley males while Long-Evans males and females had similar % PPI (Sex X Strain). In addition, Stress-induced reductions in % PPI to the 120 dB stimulus with 82 dB pre-pulse were greater for females than for males [Sex X Stress: F(1, 152)=3.92].

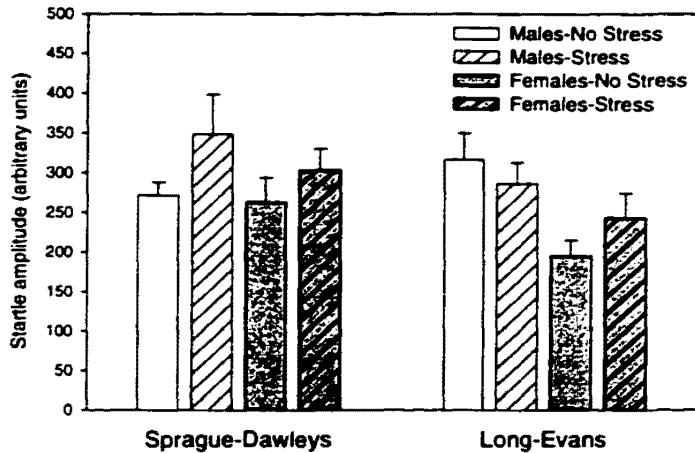
When the Strains were considered separately, there were multivariate Stress effects [F(4, 75)=3.44] among Sprague-Dawleys, with Stress decreasing % PPI to the 120 dB stimulus [82 dB: F(1, 78)=5.31] and to the 110 dB stimulus

[68 dB:  $F(1, 78)=8.74$ ; 82 dB:  $F(1, 78)=6.07$ ]. Among Long-Evans, there were no multivariate effects but Long-Evans males exhibited greater % PPI to the 120 dB stimulus with 82 dB pre-pulse than did Long-Evans females. Stress also increased % PPI to this stimulus among males but decreased % PPI among females [Sex X Stress:  $F(1, 70)=4.76$ ].

When the subgroups were considered separately, there were multivariate Stress effects among Sprague-Dawley females [ $F(4, 35)=2.97$ ] and Long-Evans females [ $F(4, 30)=2.53$ ;  $p = 0.06$ ]. For Sprague-Dawley females, Stress decreased % PPI to the 110 dB stimulus [68 dB:  $F(1, 38)=7.56$ ; 82 dB:  $F(1, 38)=10.77$ ]. For Long-Evans females, Stress decreased % PPI to the 120 dB stimulus [82 dB:  $F(1, 33)=4.22$ ]

and to the 110 dB stimulus [82 dB:  $F(1, 33)=6.35$ ]. In addition, among Sprague-Dawley males Stress decreased % PPI to the 110 dB stimulus [68 dB pre-pulse:  $F(1, 36)=3.61$ ;  $p = 0.066$ ].

There were no Stress effects within Long-Evans males.



**Figure 43.** Startle amplitude to 120 dB stimulus on Stress Day 7.

**Stress Day 7 - Startle Amplitude.** See Figures 43 and 44, and Table 43 (Appendix A). When all animals were considered together, there were multivariate Strain differences. Univariate tests indicated that Sprague-Dawleys startled more than did Long-Evans to the 110 and 120 dB stimuli. Stress also

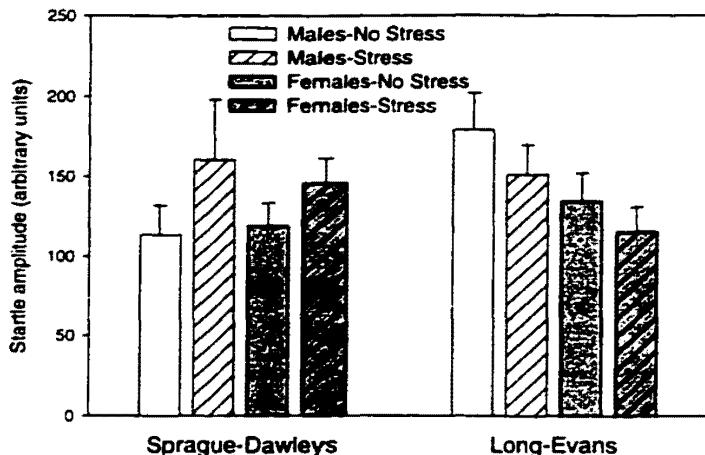
tended to increase startle to the 120 dB stimulus [ $F(1, 157)=2.96; p = 0.087$ ]. Stress increased Sprague-Dawley startle to the 110 dB stimulus but decreased Long-Evans startle [Strain X Stress:  $F(1, 157)=4.19$ ].

When the strains were

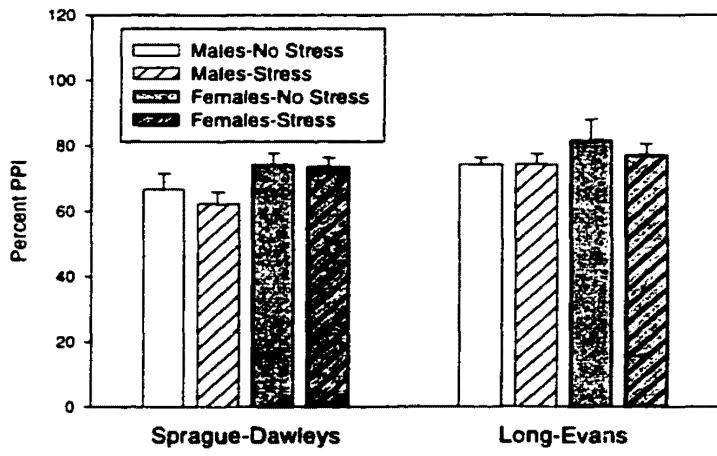
considered separately, multivariate effects were not significant. Among Sprague-Dawleys, Stress increased startle to the 120 dB stimulus [ $F(1, 81)=4.69$ ], and this increase also was evident when Sprague-Dawley males were considered alone [ $F(1, 38)=3.75; p = 0.06$ ]. Among Long-Evans, there were no Stress effects. Further analyses among Long-Evans subgroups were not done.

#### **Stress Day 7 - % PPI. See Figures 45 and 46, and Table 44 (Appendix**

A). When all animals were considered together, there were multivariate Strain and Sex differences. Univariate tests indicated that Long-Evans had greater % PPI to the 120 dB stimulus than did Sprague-Dawleys regardless of pre-pulse level. Females had greater %



**Figure 44. Startle amplitude to 110 dB stimulus on Stress Day 7.**



**Figure 45. Percent PPI to 120 dB stimulus with 82 dB pre-pulse on Stress Day 7.**

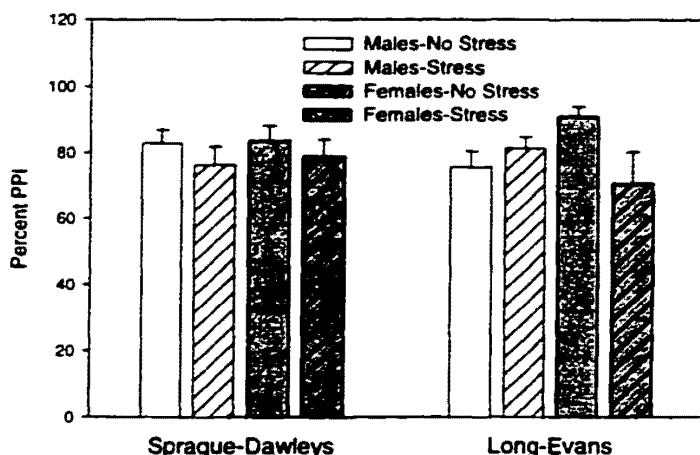
PPI to the 120 dB stimulus than did males regardless of pre-pulse level and had greater % PPI to the 110 dB stimulus with a 68 dB pre-pulse.

When the strains were considered separately, there were multivariate Sex differences within

both strains. Univariate tests indicated that Sprague-Dawley females exhibited greater % PPI to the 120 dB stimulus with 82 dB pre-pulse and to the 110 dB stimulus with 68 dB pre-pulse than did males. Long-Evans females exhibited greater % PPI than did Long-Evans males to the 120 dB stimulus regardless of pre-pulse level. Among Long-Evans, Stress decreased % PPI to the 120 and 110 dB stimuli with a 68 dB pre-pulse [120 dB:  $F(1, 71)=4.18$ ; 110 dB:  $F(1, 71)=3.59$ ].

Only Long-Evans subgroups were examined separately. There were no multivariate Stress effects. Univariate tests indicated that among Long-Evans females Stress decreased % PPI to the 120 dB stimulus with 68 dB pre-pulse [ $F(1, 33)=4.98$ ].

**Stress Day 10 - Startle Amplitude.** See Figures 47 and 48, and Table 45 (Appendix A). When all animals were considered together, there were multivariate Strain and Sex effects and a Strain X Stress interaction [ $F(2, 156)=4.67$ ]. Univariate tests indicated that Sprague-Dawleys startled more than



**Figure 46.** Percent PPI to 110 dB stimulus with 82 dB pre-pulse on Stress Day 7.

did Long-Evans to both stimuli and that males startled more than did females to the 120 dB stimulus. Stress increased startle to the 120 dB stimulus among Sprague-Dawleys but decreased it among Long-Evans [Strain X Stress:  $F(1, 157)=9.31$ ] with a tendency for the same pattern to the 110 dB stimulus [ $F(1, 157)=2.84$ ;  $p = 0.09$ ].

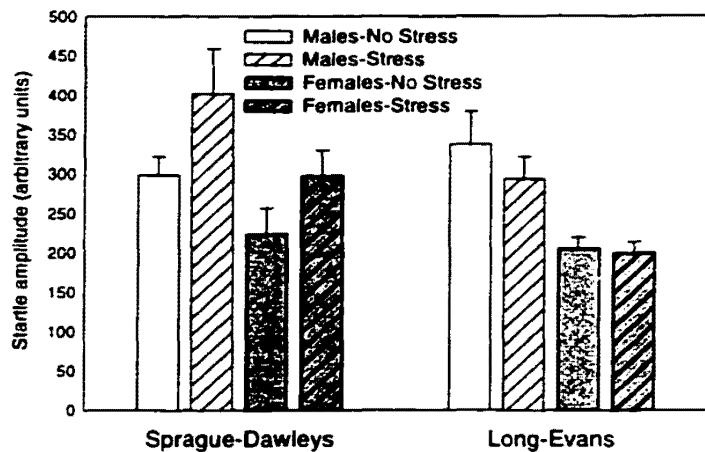
When the strains were considered separately, there was a multivariate effect of Stress among Sprague-

Dawleys [ $F(2, 80)=3.93$ ], with Stress increasing startle to both stimuli [120 dB:  $F(1, 81)=7.60$ ; 110 dB:  $F(1, 81)=3.25$ ;  $p = 0.07$ ].

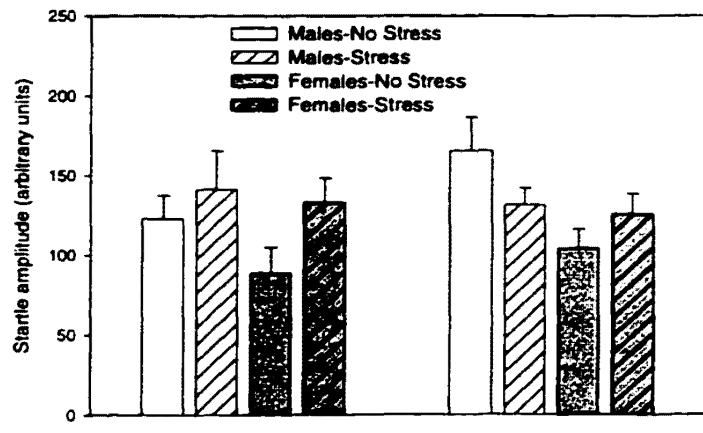
Sprague-Dawley males also tended to startle more than did Sprague-Dawley females to the 120 dB stimulus [ $F(1, 81)=3.42$ ;  $p = 0.068$ ]. Among Long-Evans, there was a trend for a

multivariate Sex effect ( $p=0.078$ ) with males startling more to the 120 dB stimulus than females.

Only Sprague-Dawley subgroups were considered separately because of the lack of



**Figure 47.** Startle amplitude to 120 dB stimulus on Stress Day 10.



**Figure 48.** Startle amplitude to 110 dB stimulus on Stress Day 10.

Stress effects among Long-Evans. For Sprague-Dawley females there was a multivariate effect of Stress [ $F(2, 40)=3.27$ ], with Stress increasing startle to both stimuli [120 dB:  $F(1, 41)=5.30$ ; 110 dB:  $F(1, 41)=5.40$ ]. Stress also increased male Sprague-Dawley startle to the 120 db stimulus [ $F(1, 38)=4.84$ ].

**Stress Day 10 - % PPI.** See Figures 49 and 50, and Table 46 (Appendix

A). When all animals were considered together, there was a multivariate trend for effects of Sex ( $p = 0.06$ ), a

multivariate effect of Stress [ $F(4, 149)=2.99$ ], and a trend for a Strain

X Stress interaction [ $F(4, 149)=2.21$ ;  $p = 0.07$ ]. Univariate

tests indicated that Long-Evans exhibited greater % PPI than did Sprague-Dawleys to the 110 dB stimulus regardless of pre-pulse

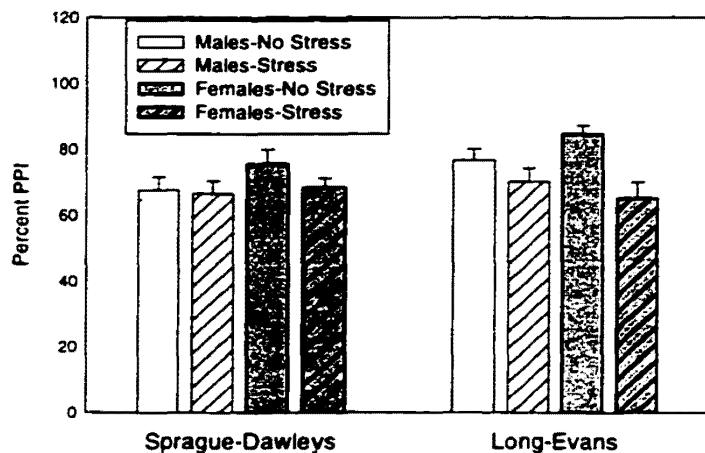
level. Females exhibited greater %

PPI than did males to both stimuli

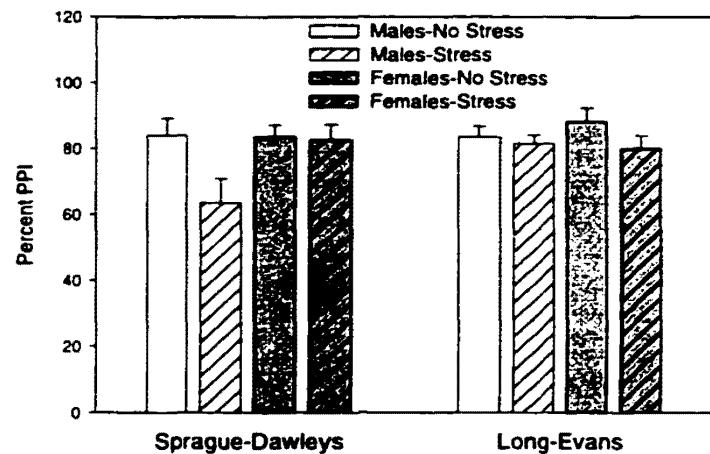
when presented with a 68 dB pre-

pulse. Stress decreased % PPI to both stimuli when presented with an

82 dB pre-pulse [120 dB:  $F(1, 152)=8.28$ ; 110 dB:  $F(1, 152)=5.31$ ], and tended to reduce



**Figure 49.** Percent PPI to 120 dB stimulus with 82 dB pre-pulse on Stress Day 10.



**Figure 50.** Percent PPI to 110 dB stimulus with 82 dB pre-pulse on Stress Day 10.

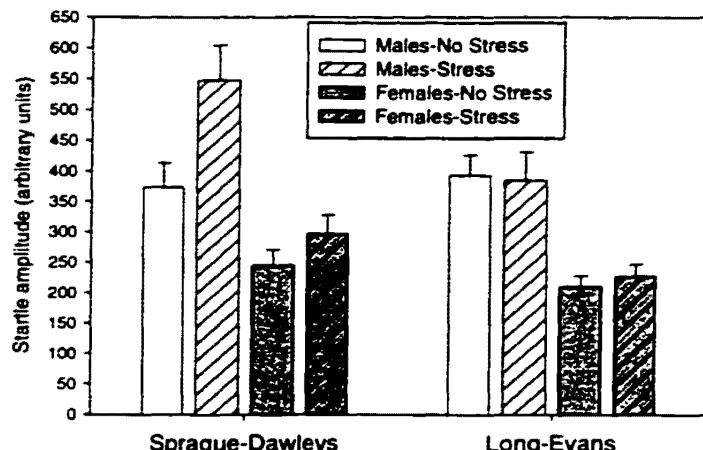
% PPI to the 120 dB stimulus with 68 dB pre-pulse [ $F(1, 152)=2.94$ ;  $p = 0.088$ ]. Stress-induced % PPI decreases were larger among Long-Evans than among Sprague-Dawleys to the 120 dB stimulus with 82 dB pre-pulse [Strain X Stress:  $F(1, 152)=4.02$ ].

When the strains were considered separately, there were no multivariate effects among Sprague-Dawleys, but univariate tests indicated that female Sprague-Dawleys exhibited greater % PPI than did Sprague-Dawley males to the 110 dB stimulus regardless of pre-pulse level. Stress also decreased % PPI among Sprague-Dawleys to the 110 dB stimulus [68 dB pre-pulse:  $F(1, 77)=4.34$ ; 82 dB pre-pulse:  $F(1, 77)=3.38$ ;  $p = 0.07$ ]. Among Long-Evans, there were multivariate Sex and Stress effects [ $F(4, 68)=3.56$ ]. Females exhibited greater % PPI than did males to the 120 dB stimulus with 68 dB pre-pulse. Stress also decreased % PPI to the 120 dB stimulus [68 dB pre-pulse:  $F(1, 71)=3.19$ ;  $p = 0.08$ ; 82 dB pre-pulse:  $F(1, 71)=12.49$ ].

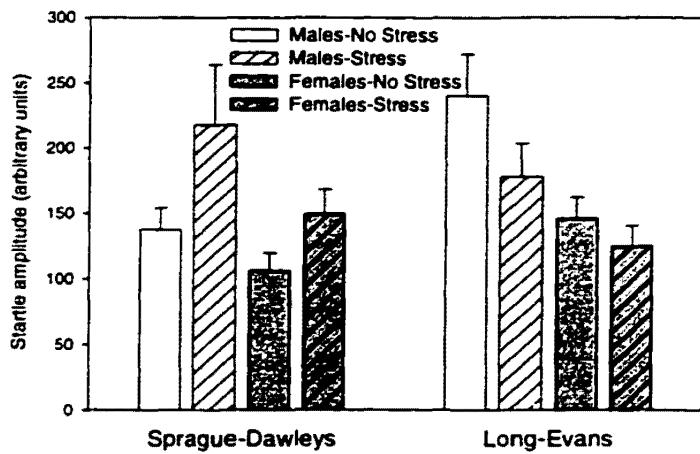
When the subgroups were examined separately, among Sprague-Dawley males Stress decreased % PPI to the 120 dB stimulus with 82 dB pre-pulse [ $F(1, 35)=3.68$ ;  $p = 0.06$ ]. Among Sprague-Dawley females, Stress decreased % PPI to the 110 dB stimulus with 68 dB pre-pulse [ $F(1, 38)=4.94$ ]. Among Long-Evans females, Stress had a multivariate effect [ $F(4, 30)=2.48$ ;  $p = 0.06$ ] and reduced % PPI to the 120 dB stimulus with 82 dB pre-pulse [ $F(1, 33)=6.71$ ]. There were no Stress effects among Long-Evans males.

**Stress Day 20 - Startle Amplitude.** See Figures 51 and 52, and Table 47 (Appendix A). When all animals were considered together, there multivariate

effects of Strain, Sex, and Stress [ $F(2, 124)=3.29$ ], and a Strain X Stress interaction [ $F(2, 124)=4.14$ ]. Univariate tests indicated that Long-Evans startled more to the 110 dB stimulus than did Sprague-Dawleys, males startled more than did females to both stimuli, and Stress increased startle to the 120 dB stimulus [ $F(1, 125)=4.66$ ]. In addition, Stress increased Sprague-Dawley startle to both stimuli but not Long-Evans startle [Strain X Stress: 120 dB:  $F(1, 125)=3.88$ ; 110 dB:  $F(1, 125)=8.26$ ].



**Figure 51.** Startle amplitude to 120 dB stimulus on Stress Day 20.



**Figure 52.** Startle amplitude to 110 dB stimulus on Stress Day 20.

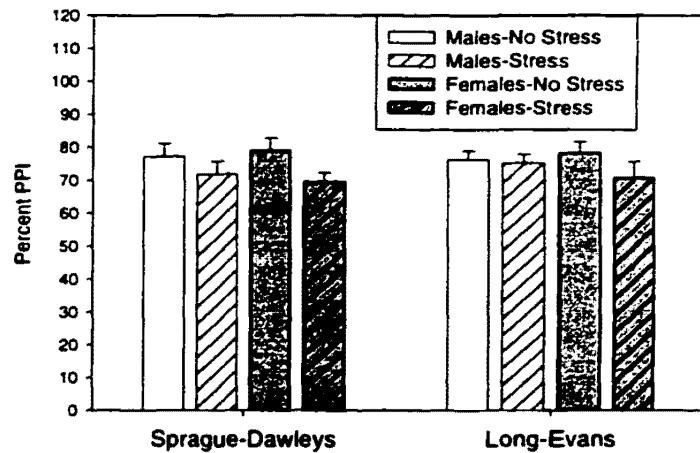
When the strains were considered separately, among Sprague-Dawleys there were multivariate Sex and Stress effects [ $F(2, 64)=3.48$ ]. Univariate tests indicated that Sprague-Dawley males startled more than did Sprague-Dawley females to both stimuli and Stress increased startle [120 dB:  $F(1, 65)=6.79$ ; 110 dB:  $F(1, 65)=4.25$ ]. Sex also interacted with Stress status with stressed males exhibiting greater startle increases to the 120 dB stimulus than stressed females

[ $F(1, 65)=4.44$ ]. Among Long-Evans, there were multivariate effects of Sex and Stress [ $F(2, 57)=3.07$ ]. Long-Evans males startled more than did Long-Evans females to the 120 dB stimulus. Stress decreased startle to the 110 dB stimulus [ $F(1, 58)=4.78$ ].

When the subgroups were considered separately, among Sprague-Dawley males there was a multivariate Stress effect [ $F(2, 29)=3.56$ ]. Stress increased startle to both stimuli [120 dB:  $F(1, 30)=6.77$ ; 110 dB:  $F(1, 30)=5.05$ ]. There were no Stress effects among Sprague-Dawley females, Long-Evans males or Long-Evans females.

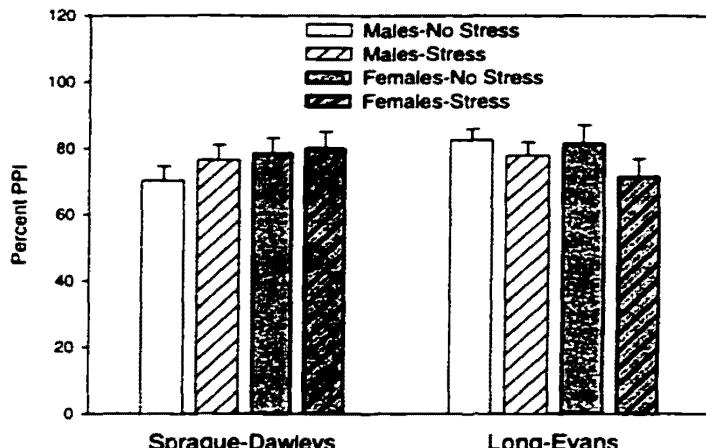
**Stress Day 20 - % PPI.** See Figures 53 and 54, and Table 48 (Appendix A). When all animals were considered together, there were weak multivariate effects of Stress [ $F(4, 118)=2.04$ ,  $p = 0.096$ ] and a weak Strain X Sex interaction. Univariate tests revealed that Long-Evans exhibited greater % PPI than did Sprague-Dawleys to the 110 dB stimulus with 82 dB pre-pulse [ $F(1, 121)=3.56$ ;  $p = 0.06$ ] and Stress decreased % PPI to the 120 dB stimulus with 82 dB pre-pulse [ $F(1, 121)=7.54$ ]. Stress also decreased % PPI to the 110 dB stimulus with 82 dB pre-pulse for Long-Evans but not for Sprague-Dawleys [Strain X Stress:  $F(1, 121)=6.16$ ].

When the strains were



**Figure 53.** Percent PPI to 120 dB stimulus with 82 dB pre-pulse on Stress Day 20.

considered separately, there were no multivariate effects among Sprague-Dawleys but Stress decreased % PPI to the 120 dB stimulus with 82 dB pre-pulse [ $F(1, 62)=4.26$ ]. Among Long-Evans, there were multivariate Sex and Stress effects [ $F(4, 52)=2.49$ ]. Stress decreased % PPI to both stimuli with the 82 dB pre-pulse [120 dB:  $F(1, 55)=3.45$ ;  $p = 0.07$ ; 110 dB:  $F(1, 55)=5.56$ ].



**Figure 54. Percent PPI to 110 dB stimulus with 82 dB pre-pulse on Stress Day 20.**

When the subgroups were considered separately, among Sprague-Dawley females there was a multivariate Stress effect [ $F(4, 27)=2.60$ ] with Stress decreasing % PPI to the 120 dB stimulus with 82 dB pre-pulse [ $F(1, 30)=7.93$ ]. There were no Stress effects for the other subgroups.

**ASR and PPI Summary.** See Tables 10 and 11 below. Effects of Stress on ASR and PPI depended on the Strain and Sex of animal and differed for each variable. Strain differences were predominant with regard to Stress effects on ASR. Specifically, Stress increased startle on every measurement day among Sprague-Dawleys, with stressed Sprague-Dawley males exhibiting increased startle throughout the experiment and stressed Sprague-Dawley females exhibiting increased startle on two of the four testing days. In contrast, Long-Evans animals exhibited increased startle only on the last testing day and this increase was not significant when Long-Evans males and females were

considered separately.

Effects of Stress on startle are relevant for interpretation of Stress effects on % PPI. Increased startle in combination with no change in % PPI would indicate no overall change in sensory-gating. Increased startle in combination with decreased % PPI or no change in startle in combination with decreased % PPI, however, would reveal a net impairment in sensory-gating.

Table 10. Statistically significant ( $p < 0.05$ ) Stress effects on ASR and PPI for each strain.						
Strain	Startle Stimulus	Pre-Pulse Stimulus	Stress Day 2	Stress Day 7	Stress Day 10	Stress Day 20
Sprague-Dawley	120 dB	None	Yes	Yes	Yes	Yes
	110 dB	None	No	No	Yes ( $p=0.07$ )	Yes
	120 dB	68 dB	No	No	No	No
	110 dB	68 dB	Yes	No	Yes	No
	120 dB	82 dB	Yes	No	No	Yes
	110 dB	82 dB	Yes	No	Yes ( $p=0.07$ )	No
Long-Evans	120 dB	None	No	No	No	No
	110 dB	None	No	No	No	Yes
	120 dB	68 dB	No	Yes	Yes ( $p=0.08$ )	No
	110 dB	68 dB	No	Yes ( $p=0.06$ )	No	No
	120 dB	82 dB	No	No	Yes	Yes ( $p=0.07$ )
	110 dB	82 dB	No	No	No	Yes

In contrast to ASR Stress effects, effects of Stress on % PPI depended on the Strain as well as the Sex of animal. Stress decreased % PPI or sensory-gating among Sprague-Dawleys and Long-Evans on most measurement days, but analyses within each sex indicated that effects were most consistent among

females of each strain. For females, therefore, Stress generally decreased sensory-gating. For Sprague-Dawley females, this decrease in sensory-gating was accompanied by an increase in startle. Together, the two changes constitute a relatively large information-processing or attentional impairment. For Long-Evans females, the decrease in sensory-gating was not accompanied by an increase in startle, indicating a moderate attentional impairment.

**Implications: Vulnerability vs. Resilience.** Females were more sensitive to stress effects on ASR and PPI than were males. Of the female subgroups, Sprague-Dawley females were the most sensitive to stress-induced impairments of sensory-gating — exhibiting increased startle as well as decreased % PPI. Long-Evans females also exhibited a net impairment but one that was not as severe, with no changes in ASR but decreases in % PPI. Males were minimally affected by stress on this measure. Sprague-Dawley males exhibited increases in ASR and occasional decreases in % PPI in response to stress. Long-Evans males were the most resistant to Stress effects on this measure, with startle and % PPI responses unaffected by Stress throughout the experiment.

These results contrast with findings from the body weight, feeding, and activity domain, in which males were most disrupted by stress. In particular, Long-Evans males were most sensitive in that domain to negative stress effects. However, in this first variable of the cognitive domain, it appears that this pattern has reversed, with females more sensitive to stress effects on ASR and PPI than males, and Long-Evans males the most stress-resistant.

**Table 11. Statistically significant ( $p < 0.05$ ) Stress effects on ASR and PPI.**

Subgroup	Startle Stimulus	Pre-Pulse Stimulus	Stress Day 2	Stress Day 7	Stress Day 10	Stress Day 20
SD Males	120 dB	None	Yes	Yes ( $p=0.06$ )	Yes	Yes
	110 dB	None	No	No	No	Yes
	120 dB	68 dB	No	No	No	No
	110 dB	68 dB	Yes ( $p=0.07$ )	No	No	No
	120 dB	82 dB	No	No	No	No
	110 dB	82 dB	No	No	Yes ( $p=0.06$ )	No
SD Females	120 dB	None	Yes	No	Yes	No
	110 dB	None	No	No	Yes	No
	120 dB	68 dB	No	No	No	No
	110 dB	68 dB	Yes	No	Yes	No
	120 dB	82 dB	No	No	No	Yes
	110 dB	82 dB	Yes	No	No	No
LE Males	120 dB	None	No	No	No	No
	110 dB	None	No	No	No	No
	120 dB	68 dB	No	No	No	No
	110 dB	68 dB	No	No	No	No
	120 dB	82 dB	No	No	No	No
	110 dB	82 dB	No	No	No	No
LE Females	120 dB	None	No	No	No	No
	110 dB	None	No	No	No	No
	120 dB	68 dB	No	Yes	No	No
	110 dB	68 dB	No	No	No	No
	120 dB	82 dB	Yes	No	Yes	No
	110 dB	82 dB	Yes	No	No	No

The next question to be answered was whether this new pattern of subgroup stress sensitivities on this pre-cognitive, non-volitional behavioral

measure predicted similar patterns on other, more complex cognitive tasks. In other words, is cognitive sensitivity to stress a global construct in which sensitivity is manifested regardless of the cognitive measure employed (i.e., will females be most disrupted in this domain and males least disrupted)? Or, do stress effects vary depending on task demands? This question is important in the context of using these behavioral responses as potential markers for specific vulnerability subtypes. In addition, the different patterns of stress sensitivity manifested by the subgroups across different domains may indicate differential underlying stress vulnerabilities.

### **Passive avoidance performance.**

Passive avoidance is a simple memory task consisting of a training day and a testing day. On the training day, each animal is placed into one chamber of the shuttlebox. After an acclimation period, a light goes on and the door to a still-dark chamber opens. When animals cross into the dark compartment, a mild footshock is delivered through the grid floor. Twenty-four hours later animals are tested using the same procedure (except that no shock is delivered if animals cross into the dark chamber). Latencies to cross into the dark chamber on the testing day are interpreted as behavioral evidence of memory, with longer latencies or not crossing into the chamber at all indicating better memory.

In this experiment, passive avoidance is conceptualized as representing a task intermediate in complexity between reflexive non-conscious ASR and PPI responses and complex search strategy and spatial memory responses required by the Morris water maze.

**Analytic approach.** Data from 11 animals out of a total of 167 (about 6.6%) were not usable. Three animals did not cross into the dark chamber during training (one Sprague-Dawley non-stress female, one Long-Evans non-stress female, and one Long-Evans stress female). Data from the remaining eight animals were not valid because of equipment or software failure (i.e., shock was not delivered, software timing malfunctioned; four Sprague-Dawley non-stress males, two Sprague-Dawley stress males, two Long-Evans non-stress

males). Although the data were not used, the 11 animals were placed in the apparatus on the testing day so that all animals had the same number of shuttlebox exposures.

Training latencies were compared with testing latencies using Wilcoxon Signed Ranks Tests (nonparametric paired t-tests) because latencies did not meet parametric test criteria (i.e., homogeneity of variance, normal distribution). Training latencies then were transformed by raising to the power of 0.295 to resolve heterogeneous variance and were analyzed with ANOVAs.<sup>3</sup> Because testing latency data were bounded (a maximum value of 300 sec), non-normally distributed, and exhibited heterogeneous variance among subgroups, these data were analyzed with Kruskal-Wallis nonparametric ANOVAs. Because maximal memory for the aversive event is indicated by the animal not crossing into the darkened chamber at all, testing latencies also were recoded into a binary format in which each animal's performance was scored as "crossed" or "did not cross." These data were analyzed with chi-squares to determine whether the proportion of animals that did not cross was significantly greater than chance for specific groups and subgroups. Test statistic values, degrees of freedom, and p values for each test are reported in Tables 49 - 51 in Appendix A. All effects and tests

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<sup>3</sup>

The choice of transformation was made based on an SPSS function that indicates the appropriate power transform for a given data set to resolve heterogeneous variance and produce a nonsignificant Levene's test. The function is accessed by going to the Explore menu, selecting Plots, choosing the Spread- vs.-Level with Levene's Test option, and selecting Power Estimation. The function produces a spread-vs.-level plot of the natural logs of the interquartile ranges against the natural logs of the medians for all cells. The plot is then used to estimate the power for a transformation to achieve equal variances in the cells.

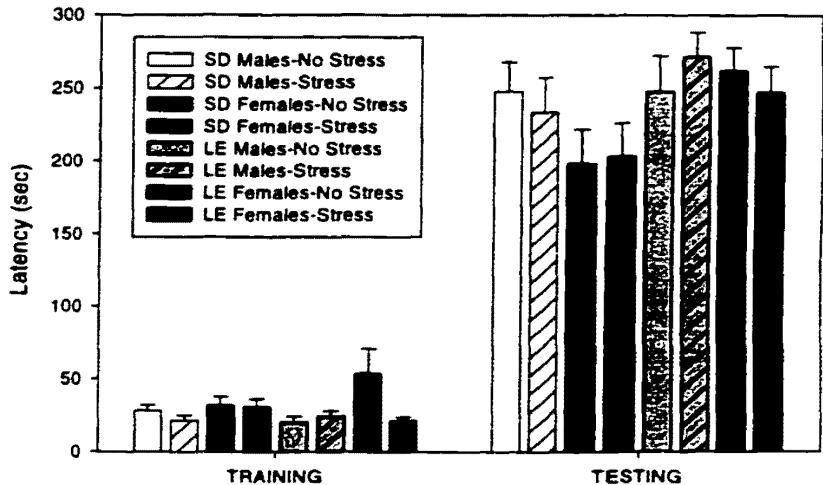
reported are significant at  $p < 0.05$  unless otherwise noted.

**Task validity.** See

Figure 55, and Table 49 (Appendix A). Before pursuing between-subjects analyses, the first issue addressed analytically was the within-subjects question of validity: did animals demonstrate memory for the aversive event that occurred

24 hrs previously in the dark chamber by either taking longer to cross into it on the test day than on the training day or by not crossing into it at all? To answer this question, training latencies were compared with testing latencies. Testing latencies were significantly longer than training latencies when all subjects were considered together ( $Z = -10.75$ ,  $df=156$ ) as well as for all subgroups, indicating that memory occurred.

**Training latencies.** See Figure 56<sup>4</sup> and Table 50 (Appendix A). When all animals were considered together, on the training day females generally took longer to cross into the dark chamber than did males [ $F(1, 148)=5.11$ ] and non-stressed animals took longer to cross than did stressed animals [ $F(1, 148)=3.30$ ,

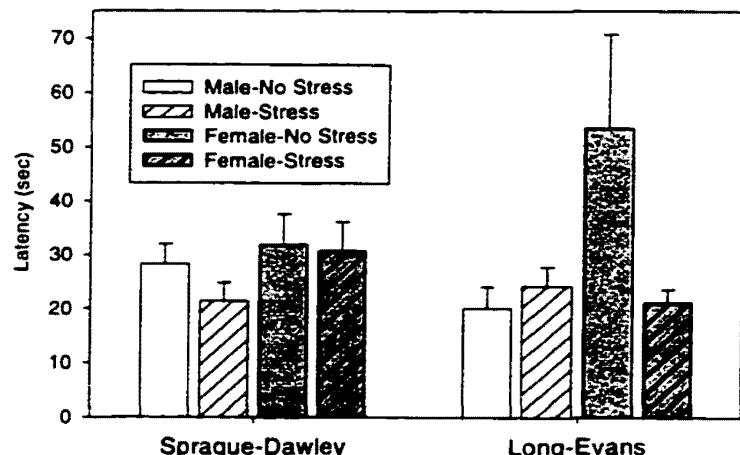


**Figure 55:** Passive avoidance training and testing latencies.

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Transformed training latencies were analyzed; raw data are graphed in Figure 56.

$p=0.07$ ]. Stress did not affect training latencies of Sprague-Dawley females and Long-Evans males but decreased training latencies for Sprague-Dawley males and Long-Evans females [Strain X Sex X Stress:  $F(1, 148)=5.91$ ].

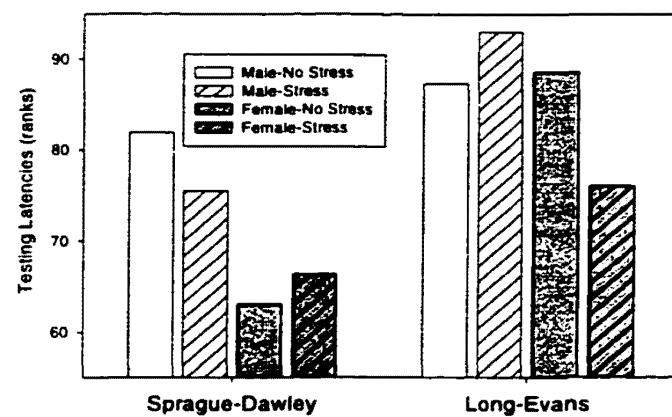


**Figure 56.** Training latencies for each treatment group.

When the strains were examined separately, among Long-Evans animals females had longer training latencies than did males [ $F(1, 72)=4.10$ ] and Stress decreased training latencies for females but not for males [Sex X Stress:  $F(1, 72)=6.41$ ]. When Long-Evans females were considered separately, Stress decreased training latencies [ $F(1, 36)=5.68$ ].

**Testing latencies. Kruskal-Wallis ANOVAs.** See Figure 57 and Table 51 (Appendix A). Latency on the testing day to enter the dark chamber is interpreted as evidence that memory has occurred, with longer latencies indicating greater memory for the aversive event 24 hrs ago.

When all animals were considered together, Long-Evans had longer test latencies than did



**Figure 57:** Mean ranks of passive avoidance testing latencies.

Sprague-Dawleys [ $\chi^2=5.90_{(1, 156)}$ ] and males had longer latencies than did females [ $\chi^2=3.38_{(1, 156)}$ ;  $p=0.06$ ]. Stress did not affect latencies. No Sex or Stress differences were revealed when the strains were examined separately.

**Chi-squares.** See Table 12 below. Because maximal memory for the task is indicated by the animal not crossing into the darkened chamber at all, comparisons between proportions of animals not crossing (i.e., animals that remembered) and crossing (i.e., animals that did not remember sufficiently) provide additional information about memory performance.

When all animals were considered together, more animals remembered (i.e., did not cross) than not (i.e., did cross). This difference was the result of Long-Evans performance, and male performance. There were no differences among Sprague-Dawleys or among all females. Comparisons within specific subgroups indicated that significant differences between the number of animals that remembered vs. did not remember were present only among Long-Evans subjects and depended on the Sex and Stress status of the animal.

Among Long-Evans males, significantly more animals remembered than did not remember, and this performance remained consistent regardless of stress status, with Stress neither improving nor impairing memory. Non-stressed Long-Evans female performance was comparable, with significantly more animals remembering than not remembering. Stress, however, disrupted Long-Evans female performance. That is, there were no differences between the number of stressed females that remembered vs. did not remember, suggesting that this subgroup's performance was at the level of chance.

**Table 12:** Number of animals that did not remember (i.e., crossed into the dark chamber) vs. remembered (i.e., did not cross) on the training day.

Group Tested	# Crossed	# Did not cross	Chi-square (df)	p value
All animals	59	97	9.256 (1,156)	p=0.002
Sprague-Dawley	37	43	0.450 (1,80)	n.s.
Long-Evans	22	54	13.474 (1,76)	p<0.001
Males	22	52	12.162 (1,74)	p<0.001
Females	37	45	0.780 (1,82)	n.s.
SD males	14	22	1.778 (1,36)	n.s.
SD females	23	21	0.091 (1,44)	n.s.
LE males	8	30	12.737 (1,38)	p<0.001
LE females	14	24	2.632 (1,38)	n.s.
SD males-No Stress	6	11	1.471 (1,17)	n.s.
SD males-Stress	8	11	0.474 (1,19)	n.s.
SD females-No Stress	11	9	0.200 (1,20)	n.s.
SD females-Stress	12	12	0.000 (1,24)	n.s.
LE males-No Stress	4	14	5.556 (1,18)	p=0.018
LE males-Stress	4	16	7.200 (1,19)	p=0.007
LE females-No Stress	5	14	4.263 (1,19)	p=0.039
LE females-Stress	9	10	0.053 (1,19)	n.s.

**Passive avoidance summary.** Overall, Long-Evans animals performed this simple memory task better than did Sprague-Dawleys regardless of whether testing latencies were analyzed as ranks or recoded into a binary format. Males also generally performed the task better than did females. Stress did not alter Sprague-Dawley performance or Long-Evans male performance, but did hinder Long-Evans female performance. This memory impairment may be related to the decreased training latencies also observed in stressed Long-Evans females. Perhaps long training latencies, during which cues are processed, are necessary

for Long-Evans females to form usable memories in this task.

**Implications: Vulnerability vs. Resilience.** With regard to stress sensitivity, Long-Evans females were the only subgroup to exhibit impaired performance on this simple memory task as a result of stress and, therefore, were the most vulnerable to stress. By comparison, the other subgroups were resistant to stress effects on this measure.

These outcomes are interesting in the context of ASR and PPI results, the other measure reported so far in the cognitive domain. Responses of Long-Evans animals were consistent across the two measures. Long-Evans males were resistant to stress effects on ASR and PPI (i.e., the subgroup did not exhibit any sensory-gating deficits as a result of stress) and also were resistant to stress effects on passive avoidance, a simple memory task. Long-Evans females exhibited moderate sensory-gating deficits (i.e., decreases in % pre-pulse inhibition without changes in ASR) in response to stress and also manifested impairment on this simple memory task.

Response of Sprague-Dawleys were not consistent across these two measures. Sprague-Dawley males exhibited minimal sensory-gating impairment as a result of stress but no impairment on passive avoidance. Sprague-Dawley females exhibited marked sensory-gating impairments in response to stress, but stress did not affect performance on this simple memory task. These findings suggest that the extent to which sensitivity to stress effects at a pre-cognitive level predict stress consequences for simple memory performance depends on the strain of animal. These findings also suggest that the strain and sex of

animal may interact with the demands of the task. That is, the some subgroups may manifest impaired sensory-gating or attentional processes in response to stress but intact simple memory (Sprague-Dawley females) while other subgroups may manifest impaired gating and attention as well as impaired simple memory (Long-Evans females). Whether this pattern holds for a considerably more complex cognitive measure — Morris water maze performance — is the next question to be answered. As with the other measures, these pattern differences may be important in terms of the extent to which they reflect specific, different underlying stress vulnerabilities.

These results also contrast with body weight, feeding, and activity measures. In that domain, male animals, and in particular, Long-Evans males, were most sensitive to stress effects. So far on the cognitive measures, it appears that females are most disrupted by stress effects, with Long-Evans females possibly the most sensitive female subgroup.

### **Morris water maze performance.**

Morris water maze performance is a complex task that was used in this experiment (Days 3 through 7) to index search strategy efficiency and spatial memory. The water maze task proceeded in two phases. Phase I of the task, conducted during Days 1 and 2 of testing, had two purposes: 1) to acclimate animals to the procedures; and 2) to determine whether the visual differences between the two strains might contribute to differences in maze performance during the second phase of testing. Phase II of the task, conducted during Days 3 through 7, constituted the search strategy efficiency and spatial learning portion of the task. On Trial 1 of Days 3 through 7, animals had to find the platform hidden beneath the water's surface (the platform is moved every day). Latency and distance swum on Trial 1, therefore, revealed search strategy efficiency, with shorter latencies and distances indicating more effective strategies. On Trial 2 of Days 3 through 7 (conducted 1 hr after Trial 1), animals had to remember where the platform was on Trial 1 and swim back to it. Trial 2 performance, therefore, revealed spatial memory, with shorter latencies and distances indicating better memory.

**Analytic approach. *Phase I.*** Acclimation was assumed to have occurred if, over several trials, animals swam more quickly and directly to the visible platform. The platform was visible on Trials 1 and 2 on Day 1 and on Trial 1 of Day 2. To determine whether animals acclimated to the testing situation, paired t-tests were used to compare Day 1's Trial 1 latency and distance with Trial 2.

Trial 1 performance on Day 1 also was compared with Trial 1 performance on Day 2. Differences between strains when the platform is visible might indicate that visual differences between albino and pigmented rats are relevant to performance when the platform was hidden. To determine whether the strains differed in this way, MANOVAs were used to evaluate between-groups differences on Trials 1 and 2 of Day 1 and on Trial 1 of Day 2.

**Phase II.** Paired t-tests comparing average Trial 1 times and distances to average Trial 2 times and distances were used to establish task validity. That is, performance on Trial 2 should generally be better than performance on Trial 1 if animals are learning where the platform is on Trial 1 and remembering where it is on Trial 2. MANOVAs were used to evaluate between-subjects differences on each day's Trial 1 and Trial 2 latencies and distances.

Ideally, repeated-measures analyses would have been conducted. On each day, however, a substantial number of animals (ranging from 15 to 22 from a total of 135) did not remain on the platform for 30 sec at the conclusion of Trial 1, and the identity of these "non-sitters" changed from day to day. The purpose of the 30 sec period on the platform is to allow animals to survey the room and encode spatial cues that will facilitate finding the platform on Trial 2.

Interpretation of Trial 2 behavior in terms of memory for animals that did not remain on the platform after Trial 1 is problematic. Therefore, for each day's data, animals that did not remain on the platform for at least 20 sec were removed from the data set (see Table 10 below). Because the identity of the non-sitters changed from day to day, patterns of missing data varied each day

and repeated-measures analyses were not feasible.

The non-sitting pattern was strongest among females (ranging from 12 to 19 out of a total of 69), and was especially marked among Long-Evans females. In particular, over the course of Days 3 through 7, only one non-stressed Long-Evans female (of a total of 16) remained on the platform every day; only 7 (of 16) stressed Long-Evans females remained on the platform every day. Because these patterns suggest that animal strain and sex affected motivation to perform the task, the number of animals remaining vs. not remaining on the platform on each day in various groups and subgroups were analyzed with chi-squares.

Test statistic values, degrees of freedom, and p values for each test are reported in Tables 52 - 71 in the Appendix A. All effects and tests reported are significant at  $p < 0.05$  unless otherwise noted. Multivariate effects are indicated with an underscored capital F (i.e., F) and have two degrees of freedom in the F ratio numerator. Univariate tests are indicated with a capital F and have 1

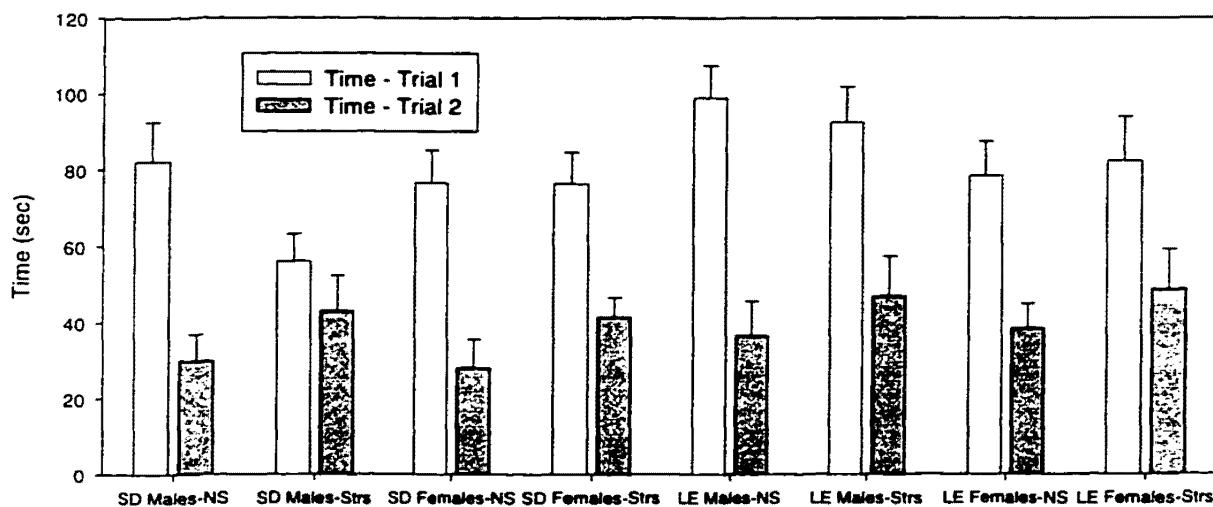
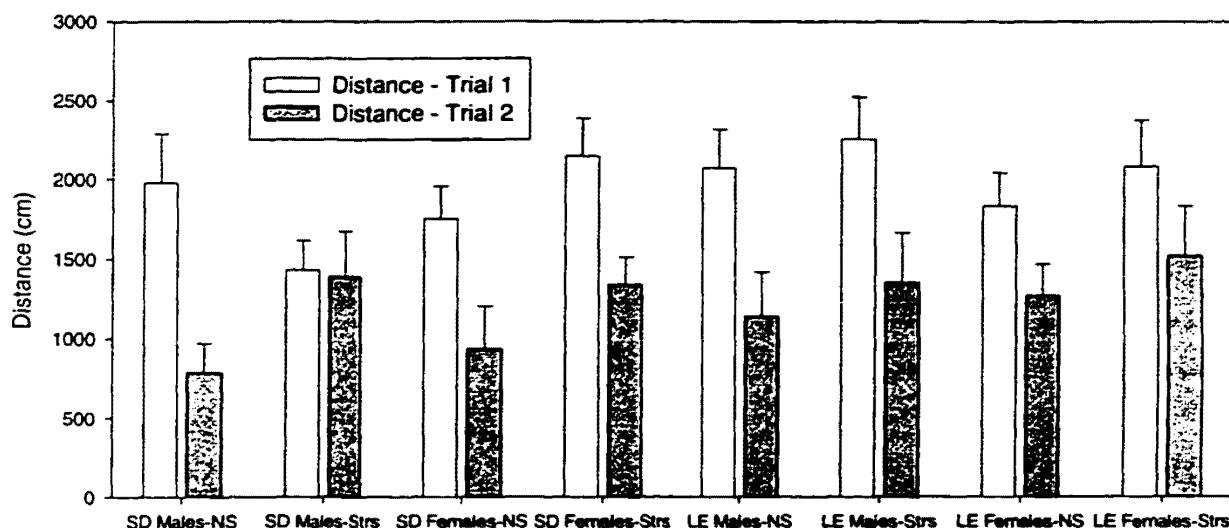


Figure 58. Latencies on Trial 1 and 2 of Day 1 for each subgroup.

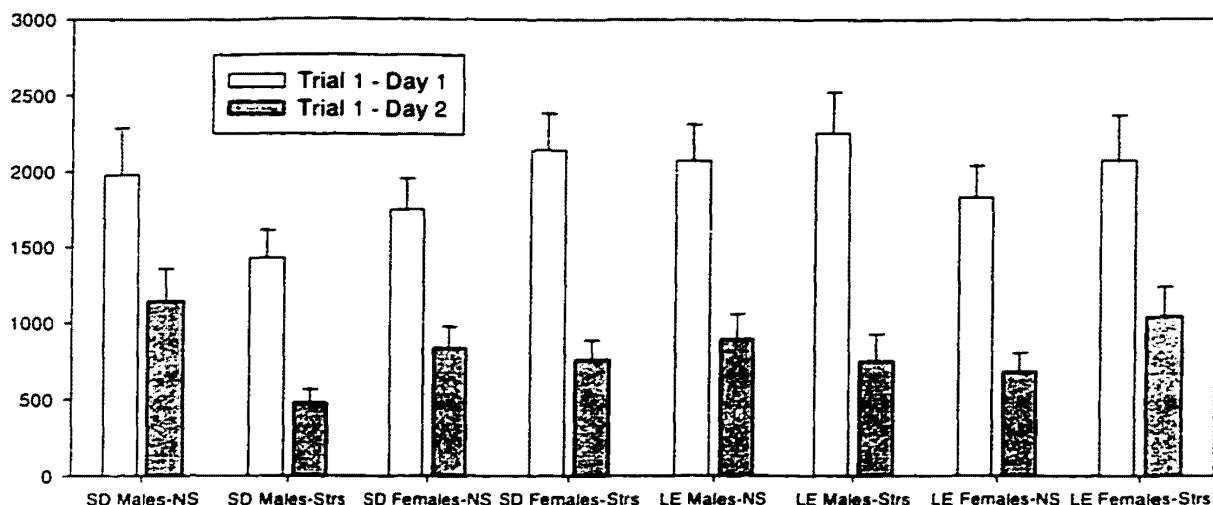
degree of freedom in the F ratio numerator.

**Phase I: Days 1 and 2. Acclimation trials.** See Figures 58 and 59, and Tables 52 and 53 (Appendix A). On Day 1, the platform was visible on both trials. Shorter latencies to climb on to the platform on Trial 2 when compared to Trial 1, or the swimming of shorter distances, would suggest that some acclimation had occurred. Paired t-tests indicated that all of the subgroups, except for stressed Sprague-Dawley males, exhibited significant improvement in time, distance, or both measures from Trial 1 to Trial 2. The lack of statistical change from Time 1 to Time 2 for stressed Sprague-Dawley males may have been the result of a floor effect because this group swam more quickly and more directly to the platform on Trial 1 than did the other groups, leaving little room for improvement on Trial 2.

Further evidence of acclimation is provided by examining latencies and distances to the marked platform on the first trial of Day 2 when compared to the



**Figure 59. Distances on Trial 1 and 2 of Day 1 for each subgroup.**



**Figure 60.** Distances on Trial 1 of Day 1 and Trial 1 of Day 2 for each subgroup.

first trial of Day 1. If animals were now acclimated to the procedure, then more rapid progress and shorter distances to the marked platform would be likely.

Paired t-tests indicated that all subgroups had shorter latencies and distances (see Figure 60; latencies are not graphed but exhibit similar patterns) on the first trial of Day 2 than on the first trial of Day 1, consistent with acclimation.

**Assessment of visual differences.** See Figures 58 and 59 above, and Table 54-57 (Appendix A). On Trial 1 of Day 1, Long-Evans rats tended to have greater latencies and distances than did Sprague-Dawley rats to the marked platform [ $F(2, 119)=2.57, p=0.082$ ] with univariate strain differences on latencies. On Trial 2 of Day 1 and Trial 1 of Day 2, there were no multivariate or univariate strain differences. No evidence emerged from trials when the platform was marked, therefore, that visual differences between the strains might influence later performance. In addition, the trend for a strain difference on Trial 1 was in the opposite direction — Long-Evans taking longer and swimming further than Sprague-Dawleys — than was hypothesized should pigmented rats have a visual

advantage.

While Strain did not reliably alter responses on the first three trials, Stress did affect subjects' latencies and distances to the marked platform. On Day 1-Trial 1, stressed animals swam more quickly and directly to the platform than did non-stressed animals [ $F(2, 119)=5.50$ ]. When the strains were considered separately, Stress improved performance of Sprague-Dawleys [ $F(2,65)=3.95$ ], but not of Long-Evans. On Day 1-Trial 2, Stress also improved performance [ $F(2,122)=2.64$ ;  $p=0.076$ ], somewhat decreasing time [ $F(1,123)=3.14$ ,  $p=0.08$ ] and decreasing distance [ $F(1,123)=4.21$ ]. When the strains were examined separately, these effects were apparent only among Sprague-Dawleys [time:  $F(1,66)=4.36$ ; distance:  $F(1,66)=4.78$ ].

On Day 2-Trial 1, Stress again improved performance of Sprague-Dawleys but not of Long-Evans [Strain X Stress:  $F(2,122)=4.89$ ] by decreasing distance, and Stress improved performance of males but not of females [Sex X Stress:  $F(2,122)=3.06$ ], decreasing time as well as distance. When the strains were examined separately, Stress improved Sprague-Dawley performance [ $F(2,62)=3.51$ ] by decreasing time and distance, and more accurately, Stress improved Sprague-Dawley male performance, not female performance [Sex X Stress:  $F(2,62)=2.50$ ,  $p=0.09$ ], reducing

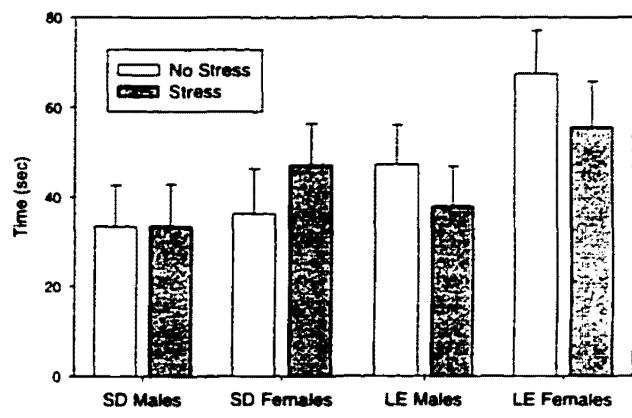
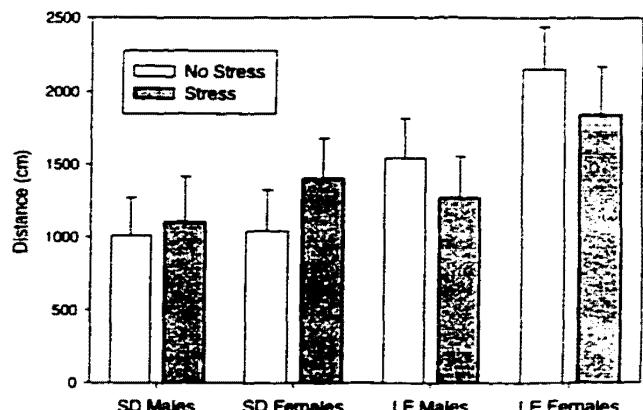


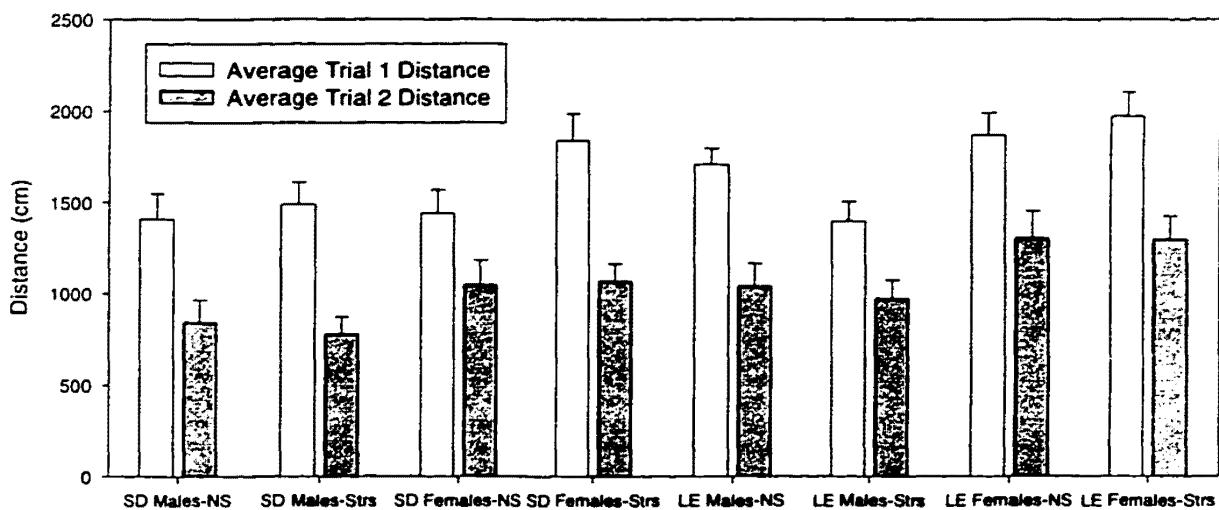
Figure 61. Latencies on Day 2-Trial 2.

distance swum by males. When Sprague-Dawley males were considered alone, these improvements in performance as a result of stress were significant as multivariate effects [ $F(2,29)=6.0$ ] and as univariate effects on time and distance.



**Figure 62.** Distances on Day 2-Trial 2.

Day 2 trials also constitute a simple memory task because the platform was marked on Trial 1 but hidden on Trial 2 with a 30 min latency between trials. When the between-groups differences were evaluated on Day 2-Trial 2 (see Figures 61 and 62), Sprague-Dawleys performed better than Long-Evans [ $F(2,126)=7.51$ ] in terms of time and distance. Males tended to perform better than females overall [ $F(2,126)=2.34$ ,  $p=0.10$ ] and on each measure. When the strains were considered separately, among Long-Evans animals males



**Figure 63.** Distances on Trial 1 and Trial 2 averaged over Days 3 to 7.

performed better than females in terms of time and distance.

### Phase II: Days 3 through 7.

**Task validity.** See Figure 63, and Table 58 (Appendix A). To make sure that animals were actually performing the task and remembering the platform location on each day's Trial 2 rather than swimming randomly about the pool, Trial 1 times and distances were averaged across Days 3 through 7 and compared with averaged Trial 2 times and distances using paired t-tests. All subgroups demonstrated significant improvement from Trial 1 to Trial 2 in terms of time as well as distance, consistent with task validity. Latencies are not graphed but exhibit patterns similar to distances.

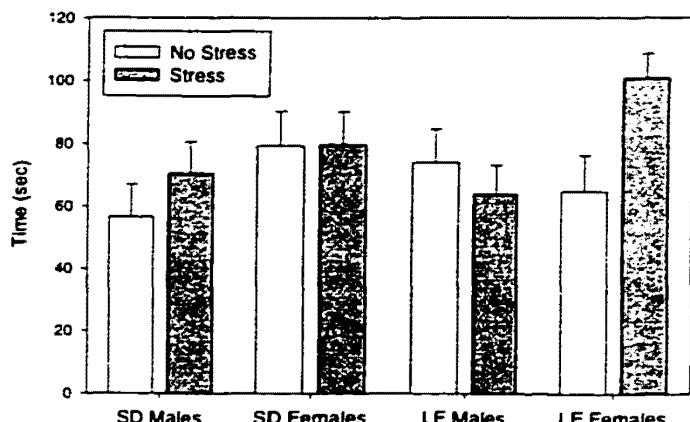


Figure 64. Time on Trial 1 of Day 3.

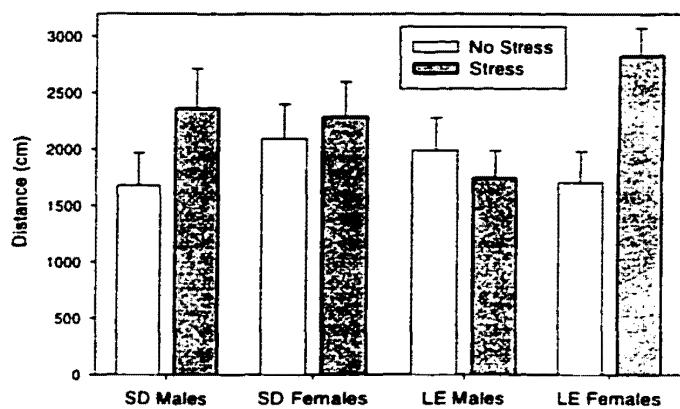


Figure 65. Distances on Trial 1 of Day 3.

**Day 3-Trial 1.** See Figures 64 and 65, and Table 59 (Appendix A). On Trial 1, males found the platform more efficiently than did females, Stress worsened performance [ $F(2, 103)=3.21$ ], and, more accurately, Strain, Sex, and Stress interacted [ $F(2, 103)=2.54, p=0.08$ ] with Stress tending to impair Sprague-Dawley male and Long-Evans female performance, and having no effect on Long-Evans males and Sprague-Dawley females. Univariate tests indicated that

males had shorter latencies than females, stressed animals swam longer distances than did non-stressed animals, and the Strain X Sex X Stress interaction was evident for time as well as distance.

When the strains were examined separately, among Sprague-Dawleys males performed better than females and Stress hampered performance [ $F(2, 52)=3.15$ ]. Univariate differences were not significant. Among Long-Evans,

Stress did not alter male performance but impaired performance of females [Sex X Stress:  $F(2, 50)=3.35$ ] in terms of time and distance. When the subgroups were examined

separately, Stress impaired performance of Sprague-Dawley

males [ $F(2, 26)=4.18$ ] and Long-

Evans females [ $F(2, 23)=4.36$ ],

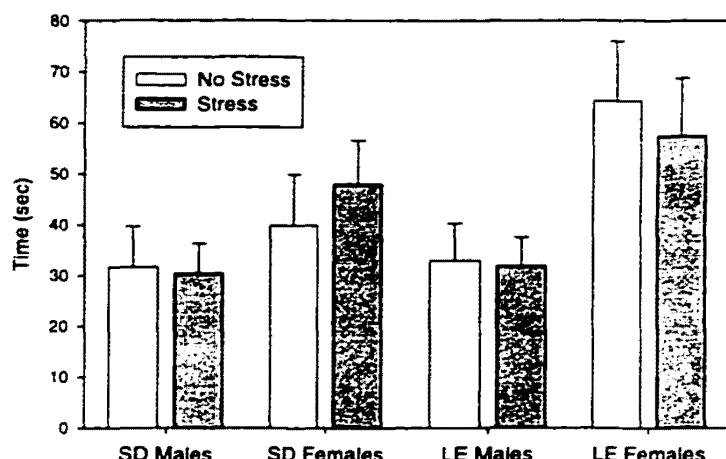
with stress increasing time and

distance for Long-Evans

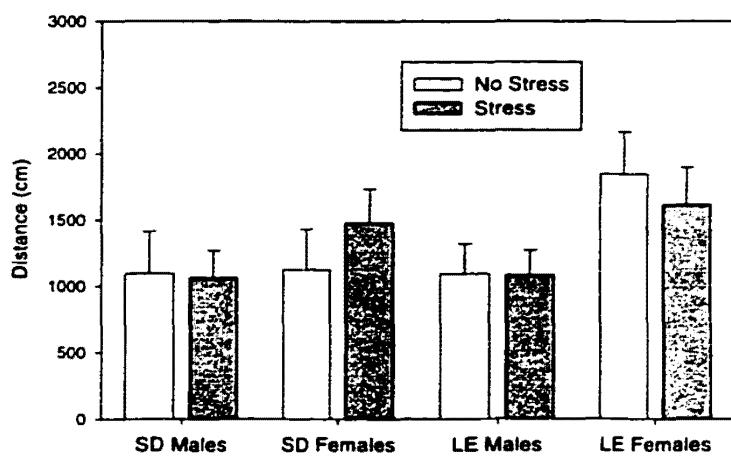
females.

**Day 3-Trial 2.** See Figures 66 and 67, and Table 60

(Appendix A). On Trial 2, males performed better than did females in terms of time as well as distance. Males also performed better than females within each



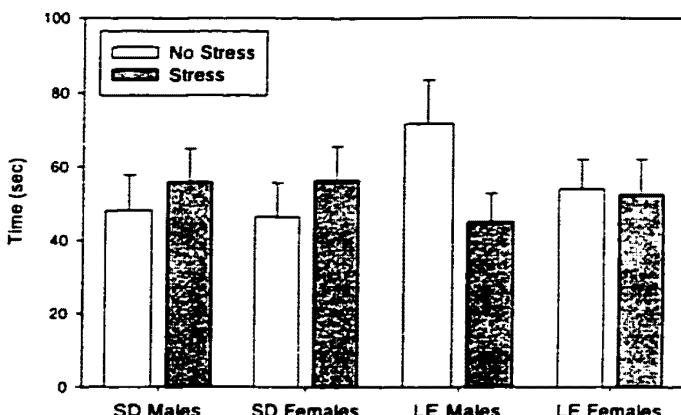
**Figure 66.** Time on Trial 2 of Day 3.



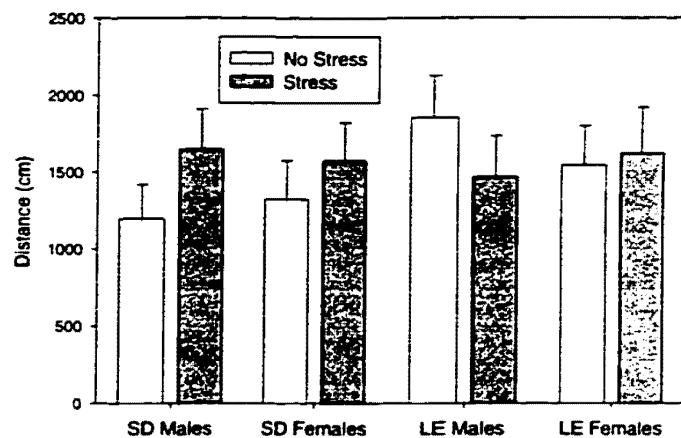
**Figure 67.** Distances on Trial 2 of Day 3.

strain, with sex differences among Long-Evans evident for time as well as distance. There were no multivariate or univariate effects of Stress. Subgroups were not examined.

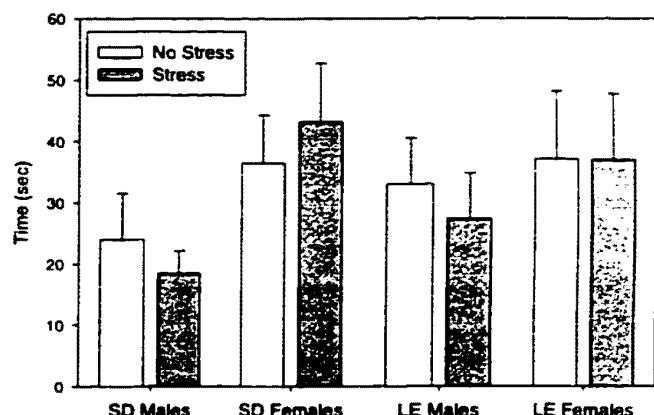
**Day 4-Trial 1.** See Figures 68 and 69, and Table 61 (Appendix A). On Trial 1, non-stressed animals performed differently from stressed animals [ $F(2, 109)=4.47$ ] but the data are best captured by the fact that Stress impaired performance of Sprague-Dawleys and improved performance of Long-Evans [Strain X Stress:  $F(2, 109)=3.63$ ], with univariate effects on time [ $F(1, 110)=3.48, p=0.06$ ]. When the strains were examined separately, Stress improved the performance of Long-Evans [ $F(2, 47)=4.76$ ] but not of Sprague-Dawleys. The improvement was the result of Long-Evans male responses [ $F(2, 28)=5.47$ ] and was evident as a univariate effect on time [ $F(1, 29)=3.74$ ,



**Figure 68.** Time on Trial 1 of Day 4.



**Figure 69.** Distances on Trial 1 of Day 4.



**Figure 70.** Time on Trial 2 of Day 4.

$p=0.06]$ .

#### **Day 4-Trial 2. See Figures**

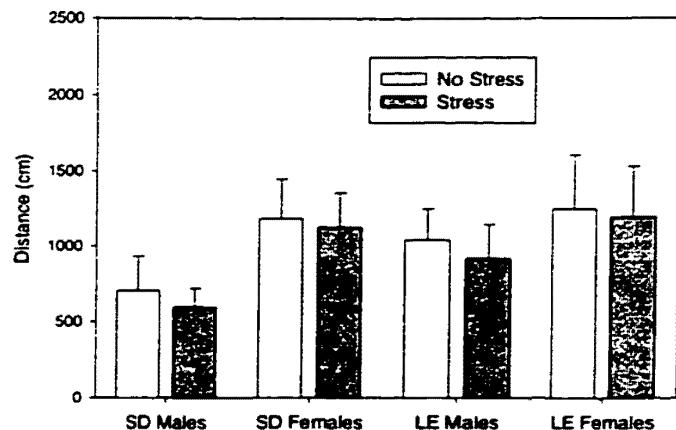
70 and 71, and Table 62 (Appendix

A). On Trial 2, Sprague-Dawleys generally performed better than did Long-Evans and Stress improved male performance but not female

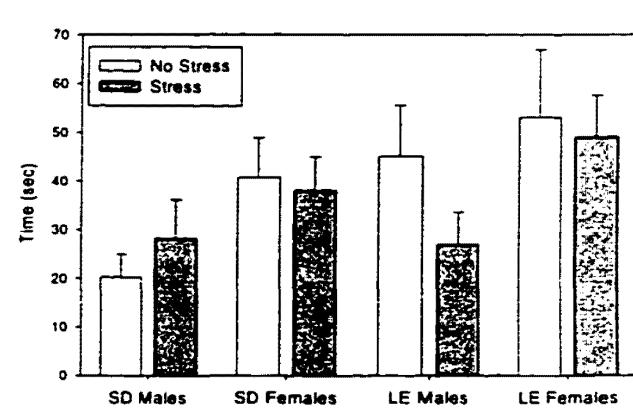
performance [Sex X Stress:  $F(2, 111)=3.29$ ]. Univariate tests indicated in addition that males performed better than did females, finding the platform faster [ $F(1, 112)=4.67$ ] and swimming shorter distances [ $F(1, 112)=4.66$ ].

When the strains were considered separately, among Sprague-Dawleys males performed better than did females [ $F(2, 62)=2.91, p=0.06$ ], with decreased times and distances, and Stress improved male performance but not female performance [Sex X Stress:  $F(2, 62)=3.36$ ]. There were no univariate Stress effects. Subgroups were not examined.

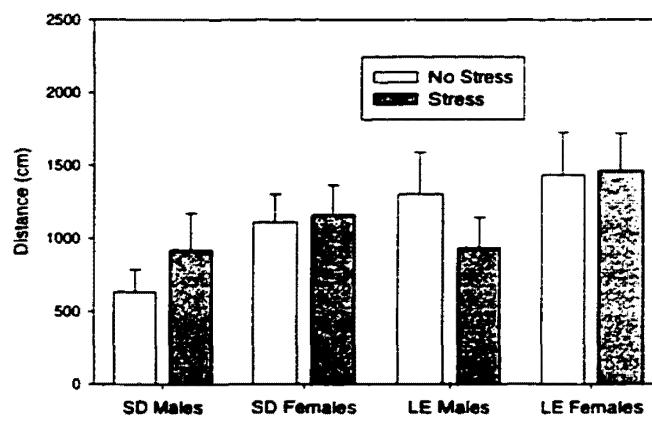
#### **Day 5-Trial 1. See Table 63**



**Figure 71. Distances on Trial 2 of Day 4.**



**Figure 72. Time on Trial 2 of Day 5.**

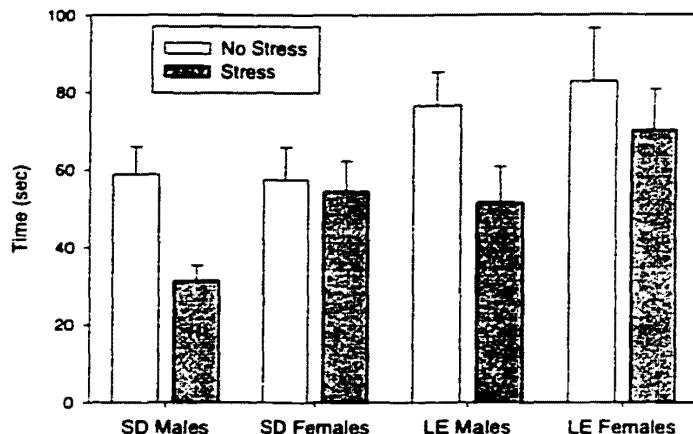


**Figure 73. Distances on Trial 2 of Day 5.**

(Appendix A). There were no between-groups differences on Trial 1 when all animals were considered together. Further analyses were not done.

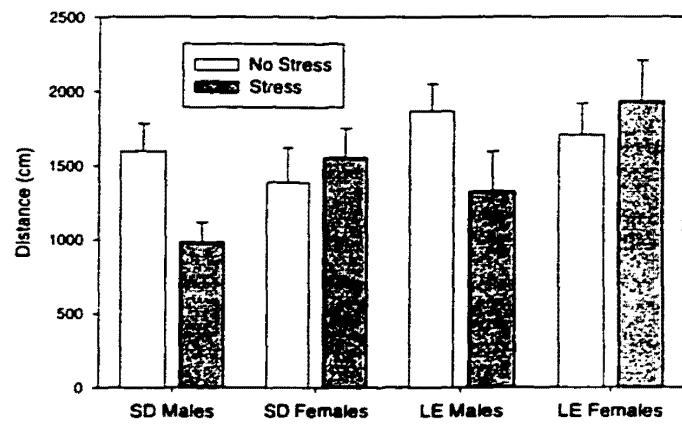
**Day 5-Trial 2.** See Figures 72 and 73, and Table 64 (Appendix A). On Trial 2, males performed better than did females, with reduced times and distances, and Stress altered performance [ $F(2, 110)=3.45$ ]. In addition, univariate tests revealed that Sprague-Dawleys took less time and swam shorter distances than did Long-Evans.

When the strains were considered separately, among Sprague-Dawleys males performed better than females in terms of time and distance ( $p=0.086$ ). Subgroups were not examined because of the lack of Stress effects.



**Figure 74.** Time on Trial 1 of Day 6.

**Day 6-Trial 1.** See Figures 74 and 75, and Table 65 (Appendix A). On Trial 1, Sprague-Dawleys performed better than Long-Evans, with shorter times and distances. Males performed better than females overall as well as in terms of time. Stress improved performance [ $F(2, 105)=12.85$ ], with univariate effects on time. More accurately,



**Figure 75.** Distances on Trial 1 of Day 6.

Stress improved performance in males but not in females [Sex X Stress:  $F(2, 105)=4.10$ ] for time as well as distance.

When the strains were considered separately, among Sprague-Dawleys Stress improved performance [ $F(2, 59)=5.96$ ] with effects on time, but more accurately Stress improved male performance, not female performance [Sex X Stress:  $F(2, 59)=2.34, p=0.10$ ] with univariate effects on time ( $p=0.06$ ) and distance. Among Long-Evans, Stress also improved performance [ $F(2, 45)=6.26$ ] with univariate effects on time.

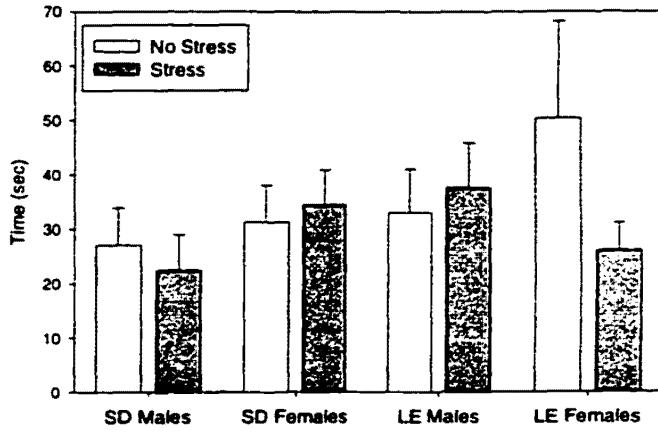
When the subgroups were examined separately, Stress improved performance of Sprague-Dawley males [ $F(2, 31)=7.21$ ], shortening time and distance. Stress also improved Long-Evans male performance [ $F(2, 28)=3.58$ ], reducing time and distance. Stress altered performance of Sprague-Dawley females [ $F(2, 27)=4.00$ ] and Long-Evans females [ $F(2, 16)=4.42$ ], slightly decreasing time to find the platform while increasing distance (nonsignificant univariate effects).

#### **Day 6-Trial 2. See Figures**

76 and 77, and Table 66

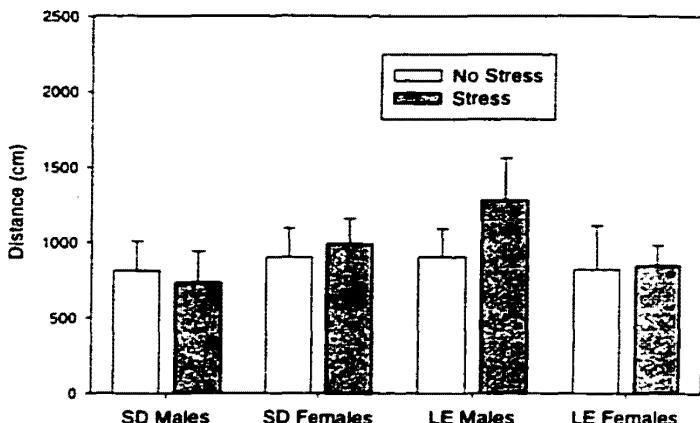
(Appendix A). On Trial 2, there were multivariate effects of Sex and Stress but the data are better explained by the interactions.

Stress altered Long-Evans performance but not Sprague-



**Figure 76. Time on Trial 2 of Day 6.**

Dawley performance [Strain X Stress:  $F(2, 105)=4.28$ ] and Sprague-Dawley males and females performed similarly while Long-Evans females performed better than Long-Evans males [Strain X Sex:  $F(2, 105)=4.52$ ].



**Figure 77.** Distances on Trial 2 of Day 6.

Stress hampered Long-Evans male performance, improved Long-Evans female performance, and had no effect on Sprague-Dawley [Strain X Sex X Stress:  $F(2, 105)=4.02$ ]. Univariate effects were not significant.

When the strains were considered separately, among Long-Evans animals females performed better than did males. Stress improved performance [ $F(2, 44)=4.80$ ], but, more accurately, Stress improved female performance and hindered male performance [Sex X Stress:  $F(2, 44)=3.04$ ]. Univariate tests were not significant. Only Long-Evans subgroups were considered separately. Stress improved performance of Long-Evans females [ $F(2, 15)=4.73$ ].

**Day 7-Trial 1.** See Figures 78 and 79, and Table 67 (Appendix A). On Trial 1, Sprague-Dawleys found the platform more efficiently than did Long-Evans in terms of time and distance. Males performed better than females, with shorter times and distances. Stress generally improved performance [ $F(2, 104)=5.15$ ] but more accurately, Stress improved Long-Evans performance and slightly impaired Sprague-Dawley performance [Strain X Stress:  $F(2, 104)=6.90$ ] in terms of time and distance. Improvements were greatest among Long-Evans

females [Strain X Sex X Stress:  $F(2, 104)=4.44$ ] for time ( $p=0.09$ ) and distance ( $p<0.05$ ).

When the strains were considered separately, among Sprague-Dawley animals males performed better than did females in terms of time as well as distance, and Stress hampered performance [ $F(2, 58)=3.12$ ], somewhat increasing time ( $p=0.09$ ) and increasing distance ( $p<0.05$ ). Among Long-Evans, males also performed better than did females in terms of time and distance but Stress improved performance [ $F(2, 45)=5.81$ ], shortening time and distance. In particular, Stress improved performance of Long-Evans females [Sex X Stress:  $F(2, 45)=3.65$ ], with univariate effects on time. When the subgroups were considered separately, this Stress-induced improvement was evident among Long-Evans females [ $F(2, 17)=7.70$ ] for time as well as distance.

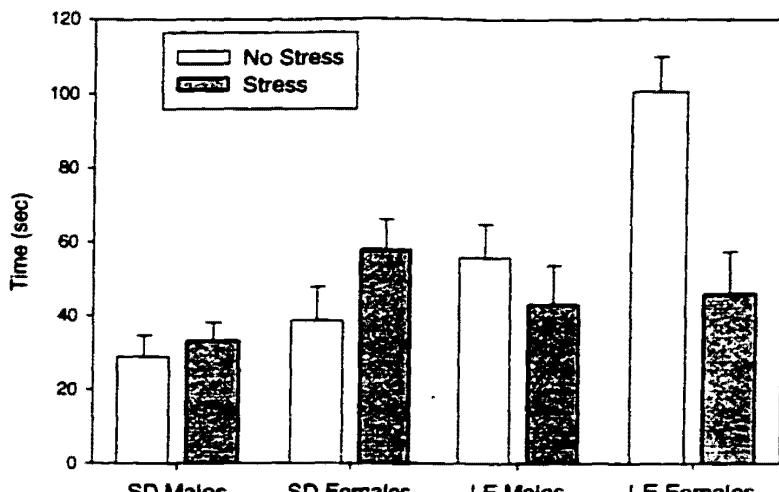


Figure 78. Time on Trial 1 of Day 7.

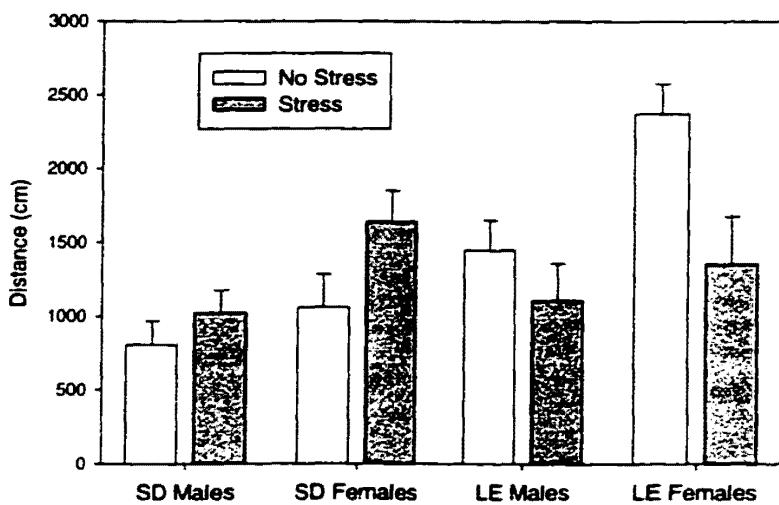


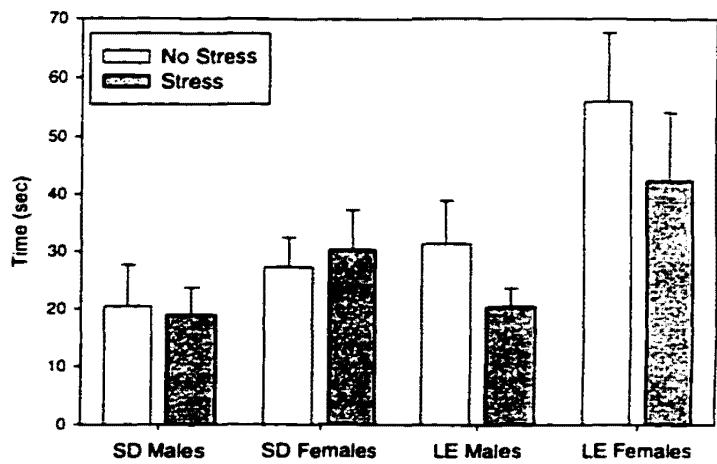
Figure 79. Distances on Trial 1 of Day 7.

**Day 7-Trial 2. See Figures 80 and 81, and Table 68 (Appendix A).**

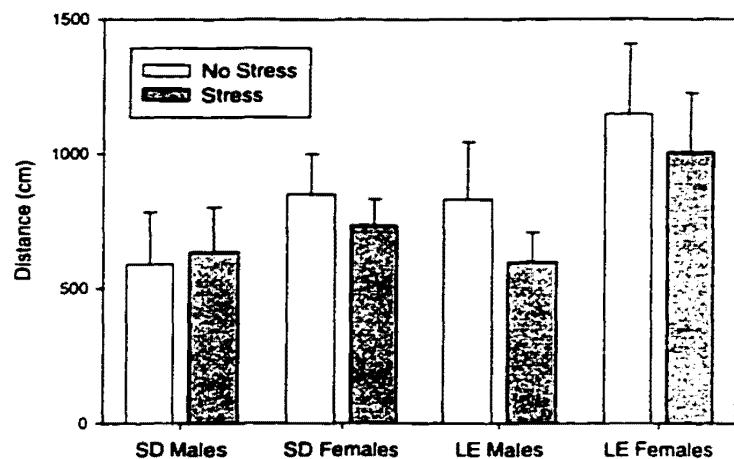
On Trial 2, Sprague-Dawleys performed better than did Long-Evans with univariate differences on time. Males also performed better than did females in terms of time and distance. When the strains were considered separately, among Long-Evans animals males performed better than did females, with shorter times and distances. Because of the lack of Stress effects, subgroups were not examined.

**Chi-squares. See Table 69**

(Appendix A). Chi-squares were performed to determine whether there were differences among groups and subgroups in the number of animals that sat on the platform for at least 20 sec at the conclusion of Trial 1 each day. For this set of analyses, the null hypothesis was that significantly more animals sat on the platform than did not sit and the tested hypothesis was that there were no differences between the number of animals that sat vs. did not sit on the platform — a reversal of the usual null hypothesis vs. tested hypothesis content in which the tested hypothesis normally is that there are differences between groups.



**Figure 80. Time on Trial 2 of Day 7.**



**Figure 81. Distances on Trial 2 of Day 7.**

Table 13 below (in which shaded blocks indicate days on which there were no differences between the number of animals sitting and not sitting in the tested group) makes clear that the non-sitting pattern was associated with the behavior of Long-Evans females. On Days 6 and 7, more than a third of Long-Evans females did not sit on the platform after Trial 1. When Long-Evans females were broken up into non-stress and stress groups, it is evident that not sitting was strongest among non-stressed Long-Evans females, with a third or more of that subgroup not sitting on each day. Stress appears to have increased the likelihood of sitting for Long-Evans females, with statistically indistinguishable numbers of animals sitting and not sitting only on Day 7 among stressed Long-Evans females.

These results suggest that motivation to perform the task differed based on animals' Sex and Strain. The methodologic and substantive implications of this possibility are addressed in the Discussion.

**Water maze summary.** Strain, Sex, and Stress status influenced search strategy efficiency and spatial memory performance. The data are best explained by the interaction of Strain, Sex, and Stress rather than by simple main effects, and these patterns also varied with day of measurement. Examination of these patterns over time helps to put the daily between-subjects differences in context.

**Table 13: Number of animals that did not sit on the platform for at least 20 sec on Trial 1.\***

<b>Group Tested</b>	<b>N</b>	<b># animals that did not sit</b>				
		<b>Day 3</b>	<b>Day 4</b>	<b>Day 5</b>	<b>Day 6</b>	<b>Day 7</b>
<b>All animals</b>	<b>135</b>	21	15	16	18	22
<b>Sprague-Dawley</b>	<b>71</b>	12	4	7	5	8
<b>Long-Evans</b>	<b>64</b>	9	11	9	13	14
<b>Males</b>	<b>66</b>	7	3	4	0	3
<b>Females</b>	<b>69</b>	14	12	12	18	19
<b>SD males</b>	<b>34</b>	4	2	3	0	1
<b>SD females</b>	<b>37</b>	8	2	4	5	7
<b>LE males</b>	<b>32</b>	3	1	1	0	2
<b>LE females</b>	<b>32</b>	6	10	8	<b>13</b>	<b>12</b>
<b>SD males-No Stress</b>	<b>17</b>	2	2	2	0	1
<b>SD males-Stress</b>	<b>17</b>	2	0	1	0	0
<b>SD females-No Stress</b>	<b>17</b>	3	1	2	1	3
<b>SD females-Stress</b>	<b>20</b>	5	1	2	4	4
<b>LE males-No Stress</b>	<b>16</b>	2	1	0	0	0
<b>LE males-Stress</b>	<b>16</b>	1	0	1	0	2
<b>LE females-No Stress</b>	<b>16</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>9</b>	<b>7</b>
<b>LE females-Stress</b>	<b>16</b>	1	4	1	4	5

\*Shaded areas indicate days on which there were no significant differences between the number of animals that sat and did not sit in the group tested.

### Search strategy (performance

on Trial 1 of Days 3-7). See Figures 82-85. For Sprague-Dawley males, Stress resulted in less efficient search strategies on Days 3 and 4, with stressed Sprague-Dawley males swimming further and longer than non-stressed males. On Day 5, however, this pattern reversed and stressed Sprague-Dawley males outperformed non-stressed Sprague-Dawley males on Days 5 and 6. On Day 7, performance of non-stressed Sprague-Dawley males further improved, resulting in no differences between the groups. For Long-Evans males, Stress generally improved search strategies, with stressed animals in this subgroup taking less time and swimming less distance than non-stressed animals on every day except for Day 5. Non-stressed Long-Evans males, however, searched less efficiently than did

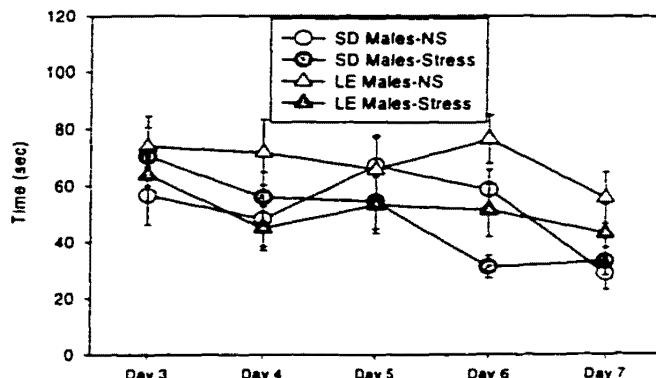


Figure 82. Trial 1 times of males.

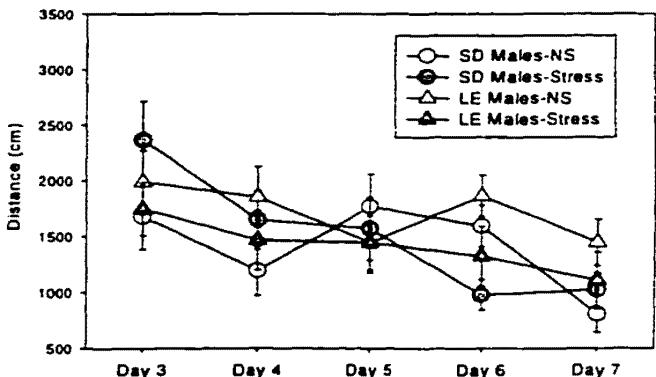


Figure 83. Trial 1 distances of males.

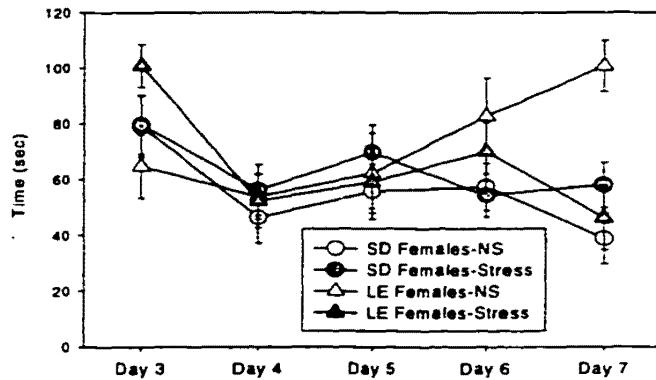


Figure 84. Trial 1 times of females.

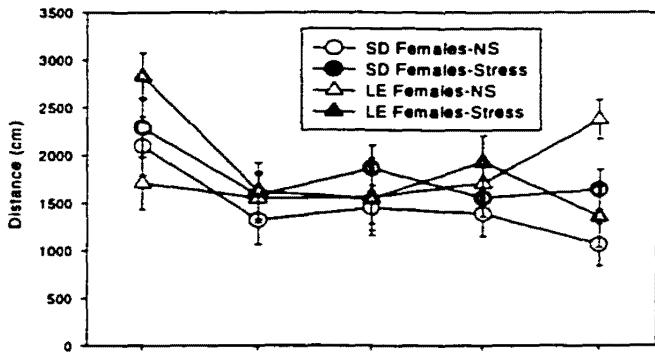
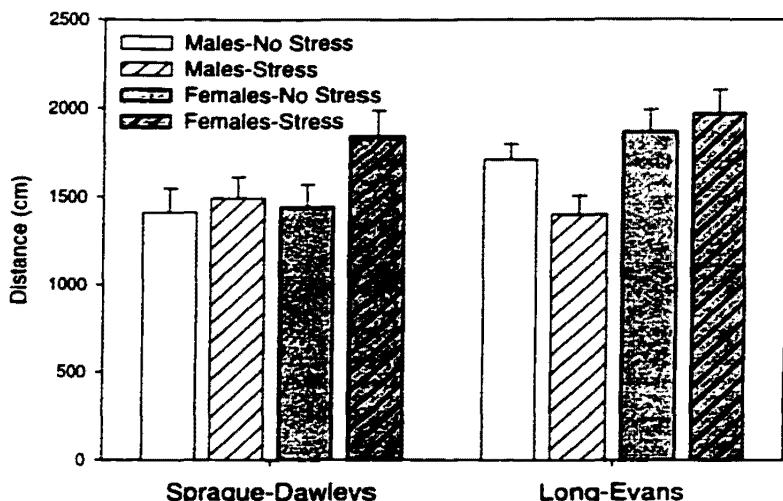


Figure 85. Trial 1 distances of females.

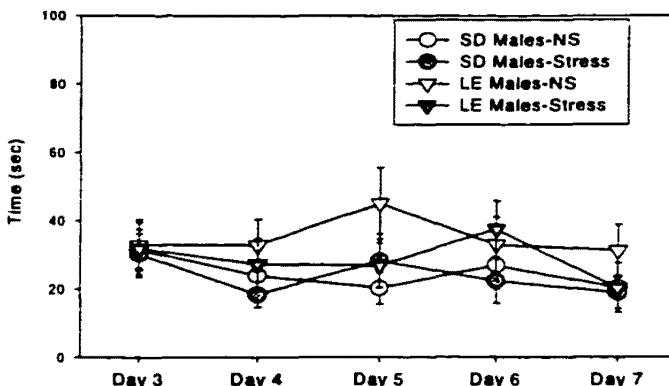
Sprague-Dawley non-stressed males, taking more time and swimming further on every day except for Day 5. This strain difference was not present among stressed males, however, which performed similarly on most days.

#### Patterns among females

were quite different. For Sprague-Dawley females Stress generally impaired search strategy, with stressed animals taking longer and swimming further to find the platform. For Long-Evans females, however, search strategies of stressed animals were initially poor and improved over time while those of non-stressed animals were initially relatively efficient but worsened over time. By Day 7, the stress-induced improvement among Long-Evans females detected statistically occurred because non-stressed animals' performance had become so poor rather than because stressed animals had markedly improved. Because of this change over time in Long-Evans female performance, non-stressed females performed similarly over Days 3, 4 and 5, but Sprague-



**Figure 86. Average Trial 1 distances over Days 3-7.**

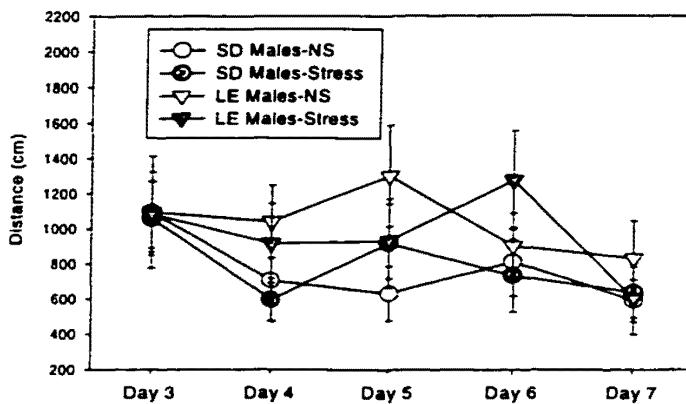


**Figure 87. Trial 2 times of males.**

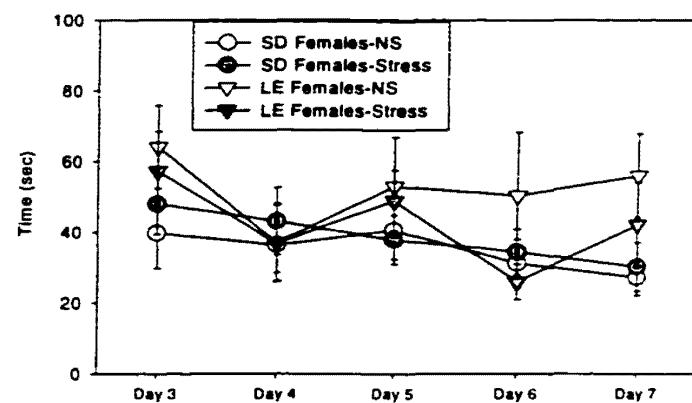
Dawley non-stressed females performed better than Long-Evans non-stressed females on Days 6 and 7. As with males, stressed females performed similarly regardless of strain.

Robust differences in search strategy — the Stress-induced improvement in Long-Evans males and the Stress-induced impairment in Sprague-Dawley females — are evident (and statistically significant) when data are averaged over the five testing days (see Figure 86 and Table 70 in Appendix A).

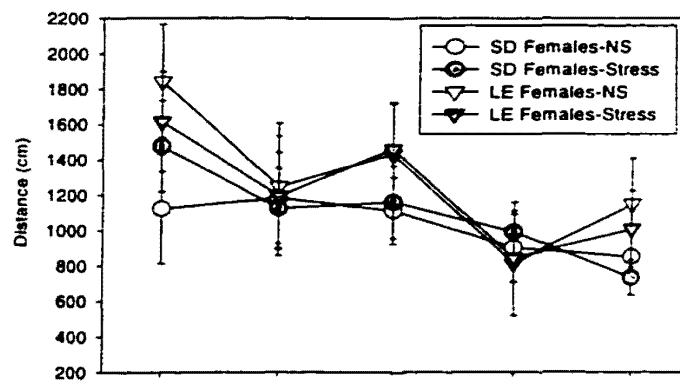
***Spatial memory***  
***(performance on Trial 2 of Days 3-7). See Figures 87-90, and Table 71 (Appendix A). There were no consistent differences as a result of Stress in spatial memory performance. Males, however, generally performed better than did females, and these sex differences were generally greater among Sprague-Dawleys than among Long-Evans.***



**Figure 88. Trial 2 distances of males.**



**Figure 89. Trial 2 times of females.**



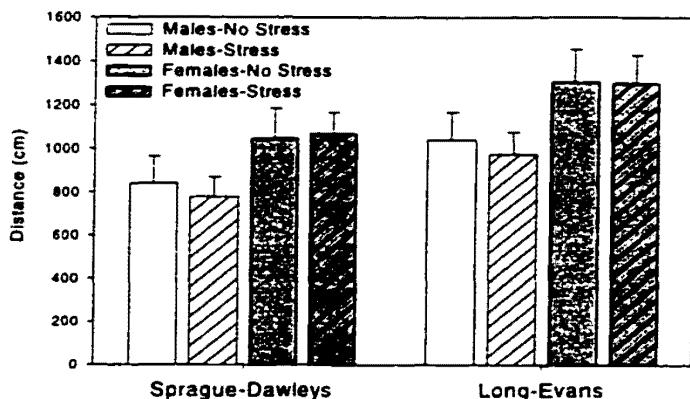
**Figure 90. Trial 2 distances of females.**

As with Trial 1 differences, robust Trial 2 differences (i.e., sex and strain differences but no Stress effects) are clear when data are averaged over the five testing days (see Figure 91).

**Implications:**

**Vulnerability vs. Resilience.** Overall, Stress altered only search strategy, improving Long-Evans male performance and impairing Sprague-Dawley female performance. Long-Evans males were most resistant, therefore, to Stress effects on this measure and Sprague-Dawley females most sensitive, with Sprague-Dawley males and Long-Evans females not consistently affected by stress.

In the context of the three cognitive domain measures (ASR and PPI, passive avoidance, and Morris water maze), maze results add complexity. The first two measures indicated that, within this domain, Long-Evans males were most stress-resistant (unaffected by stress on both measures), Sprague-Dawley males were moderately stress-resistant (stress mildly impaired sensory-gating but had no effect on simple memory performance), Sprague-Dawley females were stress-sensitive (stress markedly impaired sensory-gating but had no effect on simple memory performance) and Long-Evans females were the most stress-sensitive (with stress impairing sensory-gating as well as simple memory performance). In terms of the water maze results, Long-Evans male behaviors



**Figure 91. Average Trial 2 distances over Days 3-7.**

remained consistent. Search strategy efficiency was improved by stress in this subgroup, indicating that these animals were consistently stress-resistant across the cognitive domain. Stress impaired search strategies of Sprague-Dawley females, a finding that is consistent with the stress-induced sensory-gating impairment in this group but that diverges from the lack of stress effects on passive avoidance. To the extent that ASR and PPI results for Sprague-Dawley females indicate that stress impairs attention, it is possible that passive avoidance performance remained intact because attentional requirements of the task were minimal while the more rigorous demands of the water maze again revealed stress-induced attentional deficits in these animals.

Sprague-Dawley male maze performance was unaffected by stress, consistent with the fact that this subgroup's passive avoidance performance was unaffected by stress, and sensory-gating was only mildly impaired by stress. Long-Evans female performance is more difficult to evaluate because this subgroup appeared to lack motivation to perform the task. Tentatively, however, this subgroup's performance was unaffected by stress on this complex cognitive measure, a pattern that is not consistent with stress-induced impairments on the simpler measures (ASR and PPI and passive avoidance).

Overall, therefore, in the cognitive domain, females generally were more stress-sensitive than were males, with Sprague-Dawley and Long-Evans females exhibiting impairments on two of the three measures. Males were relatively stress-resistant in this domain, with Sprague-Dawley males exhibiting mild impairment on one measure (sensory-gating) and Long-Evans males resistant to

stress across all three measures. These findings in the cognitive domain contrast with the body weight, feeding, and activity domain, in which males, and Long-Evans males in particular, were most stress-sensitive. The sex and strain differences in stress sensitivity within and across domains imply that differential underlying stress vulnerabilities may exist within each subgroup. Given that males and females of each strain exhibited different stress sensitivity profiles depending on the domain measured, the final question to be answered was whether biochemical responses to the stressor were consistent with this diversity, or constituted a separate domain that reflected further differences in stress sensitivity.

### **Peripheral biochemical responses: Corticosterone, ACTH, and CRF.**

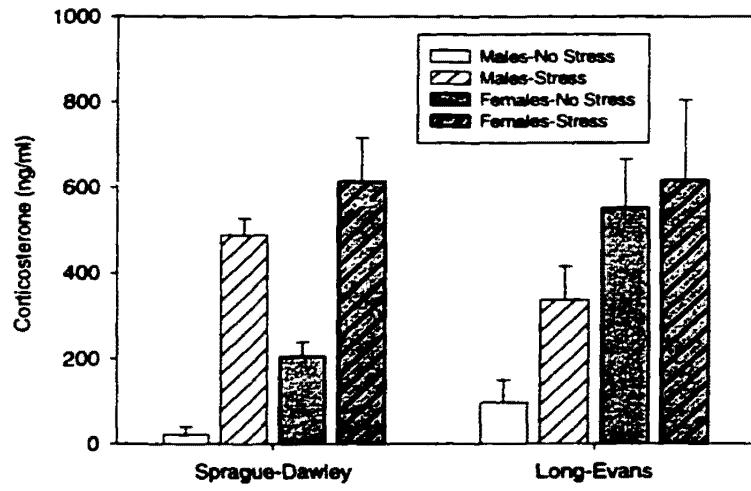
Peripheral biochemical responses were measured for two purposes: 1) to provide validation that the stressor was effective in eliciting hypothalamo-pituitary-adrenocortical (HPA) axis responses halfway through and at the end of the Stress Phase; and 2) to provide information relevant to possible mechanisms for Stress effects (i.e., feeding, body weight, cognitive performance measures).

**Analytic approach.** For the purpose of peripheral biochemical measurements, there were three cohorts of animals: 1) animals that were sacrificed halfway through the Stress Phase (n=4 per treatment cell) on Stress Day 11; 2) animals that completed the entire experiment (n=10 per treatment cell); and 3) animals that completed the experiment but were adrenalectomized 14-16 hours prior to sacrifice for the purpose of a collaboration that is not part of this doctoral dissertation (n=6 or 7 per treatment cell). Corticosterone, adrenocorticotropic hormone (ACTH), and corticotropin-releasing factor (CRF) were measured in animals that were sacrificed halfway through the Stress Phase (Cohort 1) and in animals that completed the experiment and were not adrenalectomized (Cohort 2).

Because corticosterone and ACTH data were correlated, these data from each cohort were analyzed with MANOVAs with post-hoc ANOVAs on the separate variables. CRF data were analyzed separately with ANOVAs. CRF data from four animals were not available because of problems with sample collection (one non-stress Sprague-Dawley female, one stress Sprague-Dawley female, two stress Long-Evans females).

F values, degrees of freedom, and p values for each test are reported in Tables 72 - 75 in the Appendix A. All effects and tests reported are significant at  $p < 0.05$  unless otherwise noted. Multivariate effects are indicated with an underscored capital F (i.e., F) and have two degrees of freedom in the F ratio numerator. Univariate tests are indicated with a capital F and have one degree of freedom in the F ratio numerator.

**Animals sacrificed on Stress Day 11: Corticosterone and ACTH.** See Figures 92 and 93, and Table 72 (Appendix A). Stress effects are summarized in Table 14 below. When all animals were considered together, Sprague-Dawley hormone patterns differed from Long-Evans patterns, females had higher hormone levels than did males, and Stress increased hormone levels [ $F(2, 23)=11.32$ ]. In addition, the differences between stressed and non-stressed animals were greater among Sprague-Dawleys than among Long-Evans [Strain X Stress:  $F(2, 23)=3.26$ ]. Univariate tests indicated that Sprague-Dawleys had higher ACTH levels than did Long-Evans, females had greater corticosterone levels than did males, and stressed animals had higher corticosterone and higher ACTH levels than did non-stressed animals. Differences between

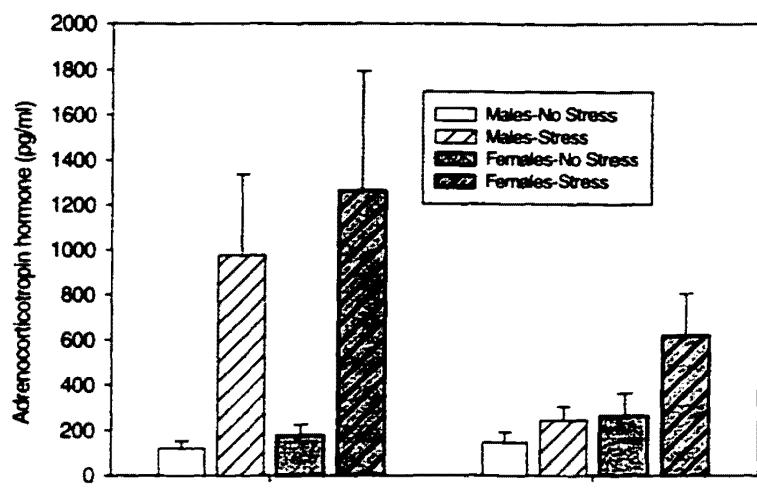


Strain	Condition	Corticosterone (ng/ml)
Sprague-Dawley	Males-No Stress	~50
	Males-Stress	~500
Sprague-Dawley	Females-No Stress	~200
	Females-Stress	~600
Long-Evans	Males-No Stress	~100
	Males-Stress	~350
Long-Evans	Females-No Stress	~550
	Females-Stress	~600

**Figure 92.** Corticosterone levels of animals sacrificed on Stress Day 11.

stressed and non-stressed animals were greater among Sprague-Dawleys than among Long-Evans for corticosterone [Strain X Stress:  $F(1, 24)=4.65$ ] as well as for ACTH [Strain X Stress:  $F(1, 24)=4.78$ ].

When the sexes were



**Figure 93.** ACTH levels of animals sacrificed on Stress Day 11.

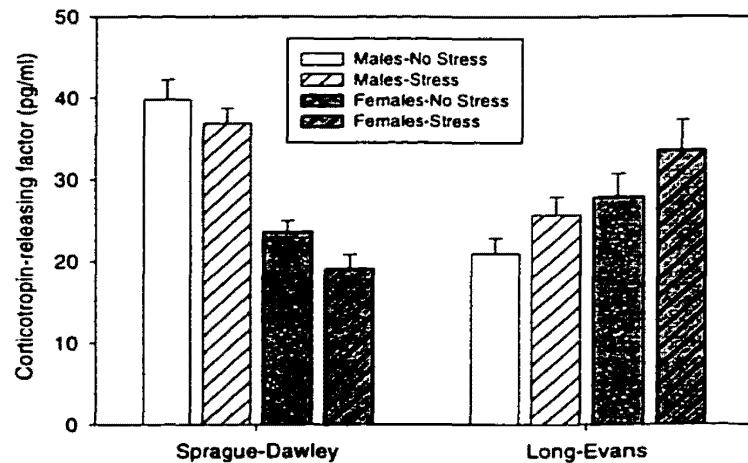
considered separately, Stress increased hormone levels among males [ $F(2, 11)=21.16$ ] and somewhat among females [ $F(2, 11)=3.40$ ;  $p=0.07$ ]. Both hormones contributed to these differences, with Stress increasing corticosterone and ACTH levels among males and among females. When the subgroups were considered separately, Stress increased hormone levels among Sprague-Dawley males [ $F(2, 5)=57.06$ ], Sprague-Dawley females [ $F(2, 5)=6.73$ ], and Long-Evans females [ $F(2, 5)=4.32$ ;  $p=0.08$ ], but not among Long-Evans males. For Sprague-Dawley males, Stress increased corticosterone and ACTH. For Sprague-Dawley females, Stress also increased corticosterone and ACTH. For Long-Evans males, although multivariate differences were absent, Stress increased corticosterone levels. Univariate tests were not significant for Long-Evans females.

**Table 14.** Statistically significant ( $p<0.05$ ) Stress effects in animals sacrificed on Stress Day 11.

Group Tested	Multivariate Effect	Corticosterone [F(df)]	ACTH [F(df)]
All animals	YES	YES [F(1, 24)=19.81]	YES [F(1, 24)=4.78]
Males	YES	YES [F(1, 12)=46.16]	YES [F(1, 12)=6.61]
Females	YES	YES [F(1, 12)=3.76]	YES [F(1, 12)=6.35]
SD Males	YES	YES [F(1, 6)=114.15]	YES [F(1, 6)=5.57]
SD Females	YES	YES [F(1, 6)=14.79]	YES [F(1, 6)=4.17]
LE Males	NO	YES [F(1, 6)=6.47]	NO
LE Females	YES	NO	NO

**Corticotropin-releasing factor (CRF).** See Figure 94 and Table 73 (Appendix A). When all animals were considered together, males had higher CRF levels than did females but the data are more clearly explained by the fact that Sprague-Dawley males had higher CRF levels than did Sprague-Dawley females while Long-Evans females had higher levels than did Long-Evans males (Strain X Sex). In addition, stressed Sprague-Dawley animals had lower CRF levels than non-stressed Sprague-Dawleys while stressed Long-Evans had higher CRF levels than non-stressed Long-Evans [Strain X Stress:  $F(1, 24)=7.11$ ].

When the sexes were considered separately, Strain differences remained and were in the opposite direction for males and females. Among



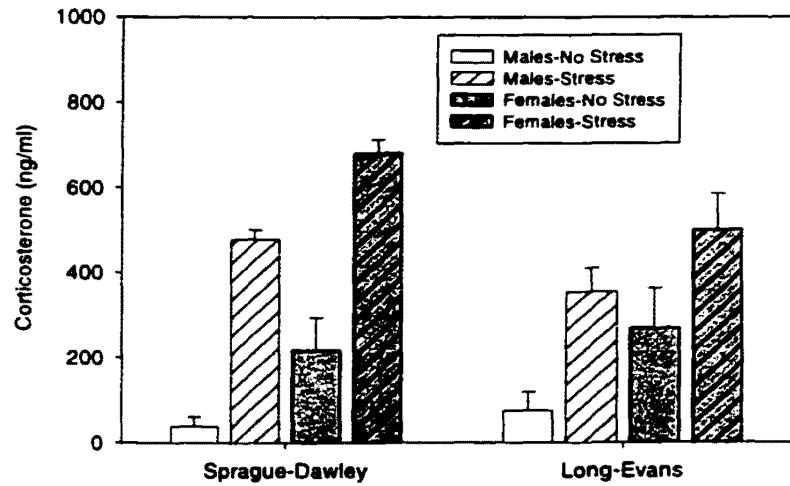
**Figure 94.** CRF levels of animals sacrificed on Stress Day 11.

males, Sprague-Dawleys had higher CRF levels than did Long-Evans; among females, Long-Evans had higher CRF levels than did Sprague-Dawleys. Strain X Stress interactions also were present within each sex, with stressed Sprague-Dawleys exhibiting lower CRF levels than non-stressed Sprague-Dawleys while stressed Long-Evans had somewhat higher CRF levels than did non-stressed Long-Evans [males:  $F(1, 12)=3.20, p=0.09$ ; females:  $F(1, 12)=3.92, p=0.07$ ]. When the subgroups were considered separately, the effects of Stress approached significance only among Sprague-Dawley females [ $F(1, 6)=4.25, p=0.08$ ] for which Stress reduced CRF.

**Animals sacrificed on Stress Day 21 (non-adrenalectomized):**

**Corticosterone and ACTH.** See Figures 95 and 96, and Table 74 (Appendix A). Stress effects are summarized in Table 15 below. When all animals were considered together, females generally had higher hormone levels than did males [ $F(2, 71)=12.43$ ] and Stress increased hormone levels [ $F(2, 71)=34.74$ ]. In addition, sex differences

in hormone levels were greater among Sprague-Dawleys than among Long-Evans [Strain X Sex:  $F(2, 71)=5.03$ ] and differences between stressed and non-stressed animals also were greater among Sprague-

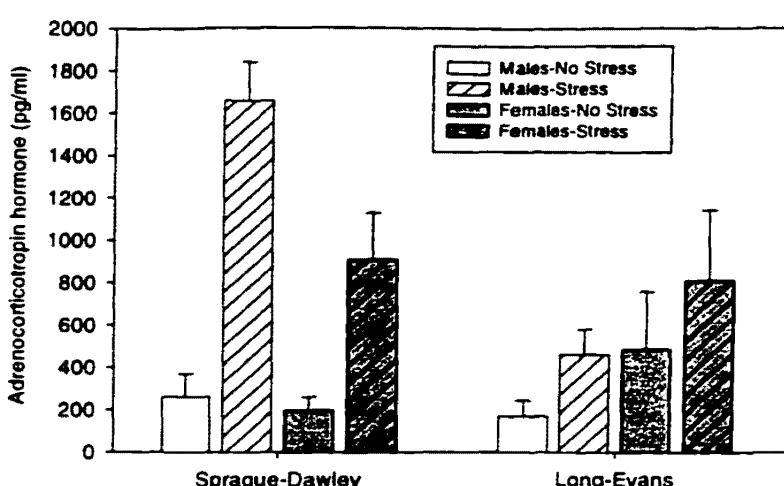


**Figure 95.** Corticosterone levels of animals sacrificed on Stress Day 21.

Dawleys than among Long-Evans [Strain X Stress:  $F(2, 71)=4.30$ ]. Univariate tests indicated that females had higher corticosterone levels than did males [ $F(1, 72)=18.21$ ] and stressed

animals had higher levels of

both hormones than did non-stressed animals. In addition, Sprague-Dawley males had higher ACTH levels than did Sprague-Dawley females while Long-Evans females had higher levels than did Long-Evans males [Strain X Sex:  $F(1, 72)=7.14$ ]. Differences between stressed and non-stressed animals also were greater among Sprague-Dawleys than among Long-Evans on both hormones [Strain X Stress; corticosterone:  $F(1, 72)=5.35$ ; ACTH:  $F(1, 72)=7.41$ ].



**Figure 96. ACTH levels of animals sacrificed on Stress Day 21.**

When the sexes were considered separately, among males Sprague-Dawleys exhibited higher hormone levels than Long-Evans [ $F(2, 35)=13.23$ ] and stressed animals had higher hormone levels than did non-stressed animals [ $F(2, 35)=45.80$ ]. In addition, the differences between stressed and non-stressed animals were greater among Sprague-Dawley males than among Long-Evans males [Strain X Stress:  $F(2, 35)=9.25$ ]. Univariate tests indicated that Sprague-Dawley males had higher ACTH levels than did Long-Evans males and stressed males had higher corticosterone and ACTH levels than did non-stressed males. The differences between stressed and non-stressed animals were greater

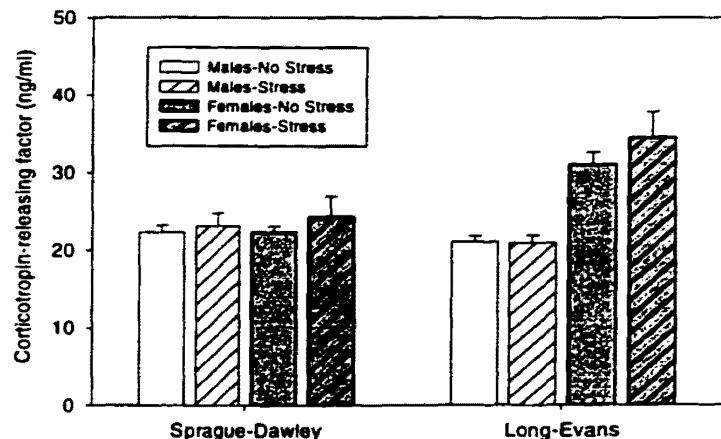
among Sprague-Dawleys than among Long-Evans for corticosterone as well as for ACTH. Among females, only multivariate effects of Stress were present [ $F(2, 35)=10.31$ ], and stressed females had higher levels of corticosterone and ACTH than did non-stressed females.

When the subgroups were considered separately, multivariate Stress effects were present among Sprague-Dawley males [ $F(2, 17)=91.84$ ], Sprague-Dawley females [ $F(2, 17)=16.50$ ], and Long-Evans males [ $F(2, 17)=7.36$ ], but not among Long-Evans females. Among Sprague-Dawley males and females, and Long-Evans males, Stress significantly increased corticosterone as well as ACTH. Among Long-Evans females, Stress increased only corticosterone.

**Corticotropin-releasing factor (CRF).** See Figure 97 and Table 75 (Appendix A). When all animals were considered together, females had higher CRF levels than did males [ $F(1, 68)=27.31$ ] and Long-Evans had higher levels than did Sprague-Dawleys [ $F(1, 68)=10.88$ ]. The data are more accurately explained, however, by the fact

that Long-Evans females had much higher levels of CRF than Long-Evans males, while Sprague-Dawley males and females had similar CRF levels [Strain X Sex:  $F(1, 68)=21.98$ ].

When the sexes were considered separately, the



**Figure 97.** CRF levels of animals sacrificed on Stress Day 21.

greater CRF levels among Long-Evans females compared to Sprague-Dawley females were clear [ $F(1, 32)=18.91$ ]. There were no differences among males. Because there were no Stress effects within males or females, subgroups were not examined.

**Table 15.** Statistically significant ( $p<0.05$ ) Stress effects in animals sacrificed on Stress Day 21.

Group Tested	Multivariate Effect	Corticosterone [ $F(df)$ ]	ACTH [ $F(df)$ ]
All animals	<b>YES</b>	<b>YES [<math>F(1, 72)=69.30</math>]</b>	<b>YES [<math>F(1, 72)=24.50</math>]</b>
Males	<b>YES</b>	<b>YES [<math>F(1, 36)=85.51</math>]</b>	<b>YES [<math>F(1, 36)=44.16</math>]</b>
Females	<b>YES</b>	<b>YES [<math>F(1, 36)=21.17</math>]</b>	<b>YES [<math>F(1, 36)=4.50</math>]</b>
SD Males	<b>YES</b>	<b>YES [<math>F(1, 18)=192.67</math>]</b>	<b>YES [<math>F(1, 18)=43.39</math>]</b>
SD Females	<b>YES</b>	<b>YES [<math>F(1, 18)=31.84</math>]</b>	<b>YES [<math>F(1, 18)=9.80</math>]</b>
LE Males	<b>YES</b>	<b>YES [<math>F(1, 18)=15.48</math>]</b>	<b>YES [<math>F(1, 18)=4.33</math>]</b>
LE Females	NO	<b>YES [<math>F(1, 18)=3.33</math>]</b>	NO

#### **Summary of peripheral hormone data. Effects through Stress Day**

11. Samples from animals sacrificed on Stress Day 11 indicated that corticosterone and ACTH responses to Stress were intact halfway through the Stress Phase, with greater responses by Sprague-Dawley animals. In particular, Sprague-Dawley males and females responded robustly to the stressor on both indices, with Stress accounting for 95% and 48% of corticosterone and ACTH variance, respectively, in males and 71% and 41% of the variance, respectively, in females. Responses were present but less robust among Long-Evans. For males, Stress significantly increased corticosterone (52% of variance) and nonsignificantly increased ACTH (21% of variance). For Long-Evans females,

Stress exerted a significant multivariate effect without significant univariate effects (1.4% of corticosterone variance and 32% of ACTH variance). The lack of univariate effects among Long-Evans females was the result of two circumstances.

First, one of the four stressed Long-Evans females had a corticosterone level of 61.7 ng/ml and an ACTH level of 82.4 pg/ml while the other three stressed animals had corticosterone levels greater than 700 ng/ml and ACTH levels greater than 690 pg/ml. The single animal's low hormonal levels reduced the stressed group's mean substantially. It is not clear why this animal's hormone levels were so low. Low values across two different assays suggest, however, that the values are accurate and not an artifact of assay procedure. It is possible that this animal always was a low responder to the stressor or, alternatively, that she habituated by Day 11.

Second, three of the four non-stressed Long-Evans females had corticosterone levels greater than 520 ng/ml but relatively low and appropriate ACTH responses. The relatively high corticosterone levels in these "non-stressed" animals resulted in a group mean that was similar to the stressed group. It is possible that the non-stressed Long-Evans females exhibited increased corticosterone levels in response to the unusual environmental event of the experimenter repeatedly entering the housing room during the light portion of the cycle. Up until this point in the experiment, animals had been left undisturbed once the lights went on. Relatively low ACTH levels would be consistent with this hypothesis because the mildly stressful event would have

elicited brief surges in CRF and ACTH that would have tapered off by the time samples were taken. Because corticosterone is the downstream product of these substances, sufficient time may not have elapsed for corticosterone to return to basal levels.

CRF results indicated that the two strains responded to Stress in opposite directions after 11 days of exposure to the stressor, with Stress decreasing Sprague-Dawley CRF but increasing Long-Evans CRF. CRF responses, therefore, were intact after 11 days of immobilization. Whether these different patterns are the result of truly opposite responses to Stress is not clear. Other explanations (i.e., strain differences in rates of CRF synthesis, metabolism, or distribution) also could account for these findings and will be addressed in the Discussion. In any case, effect sizes were notable, with Stress accounting for 13% of Sprague-Dawley male variance, 41% of Sprague-Dawley female variance, 30% of Long-Evans male variance, and 20% of Long-Evans female variance. In addition, CRF results revealed sex differences within each strain of opposite direction, with Sprague-Dawley males having higher CRF levels than Sprague-Dawley females but Long-Evans females having higher CRF levels than Long-Evans males.

***Effects through Stress Day 21.*** Samples from animals sacrificed on Stress Day 21 indicated that corticosterone and ACTH responses to Stress also were intact at the end of the Stress Phase and responses of Sprague-Dawleys again were larger than those of Long-Evans. In particular, Sprague-Dawley males and females responded robustly to the stressor on both indices, with

Stress accounting for 92% and 71% of corticosterone and ACTH variance, respectively, in males and 64% and 35% of the variance, respectively, in females.

Responses to stress were evident but less robust among Long-Evans animals. For males, Stress significantly increased corticosterone (46% of variance) and ACTH (19.4% of variance). For Long-Evans females, Stress significantly increased corticosterone (15.6% of variance) and nonsignificantly increased ACTH (3% of variance).

CRF results differed markedly from those obtained after 11 days of Stress. After 21 days of Stress exposure, effects of Stress were no longer evident (reflected by non-significant proportions of explained variance that ranged from 0.01% to 6%). These findings suggest that CRF responses had habituated to the stressor after three weeks of exposure. In addition, the Strain X Sex interaction present on Stress Day 11, in which Sprague-Dawley males and Long-Evans females exhibited higher CRF levels than Sprague-Dawley females and Long-Evans males, disappeared. CRF levels were similar across subgroups (because Sprague-Dawley male levels had dropped) with the exception of Long-Evans female CRF levels, which remained high.

**Implications: Vulnerability vs. Resilience.** All of the subgroups by hormonal indices appear to have experienced the immobilization procedure as stressful. Hormonal responses of the subgroups are not easily cast in terms of stress sensitivity, however, because response magnitude on these measures can be interpreted in different ways. For example, the robust Sprague-Dawley

corticosterone and ACTH responses to stress could be interpreted as adaptive HPA axis functioning when confronted repeatedly with a stressor to the extent that HPA responses are necessary for effective behavioral coping. On the other hand, failure to habituate to repeated stressor presentation has been identified as a problematic pattern (e.g., McEwen, 1998). According to this interpretation, the less robust Long-Evans responses would be more adaptive. The methodology of the present experiment makes this a difficult question to resolve. These issues are addressed in detail in the Discussion.

In any case, the hormonal responses do not appear to be related to data from the other domains in a simple way. For example, all of the subgroups exhibited increased corticosterone in response to stress, but stress was associated with feeding decreases in males and not in females, and with cognitive impairments in females but not in males. The magnitude and directionality of subgroup behavioral differences in response to stress, therefore, do not appear to be reflected in the HPA axis hormones. These results suggest that: 1) behaviors are important variables to distinguish subpopulations that may differ in underlying stress vulnerability; and 2) hormones may substantiate that a stressful experience has occurred without necessarily indicating in what domain behavioral changes will appear that index stress vulnerability. These issues also are discussed in more detail in the Discussion.

## **CONFIRMATION OF HYPOTHESES**

**Hypothesis 1:** The hypothesis that stress would reduce male body weight regardless of strain, and reduce Long-Evans female body weight, but have no effect on Sprague-Dawley female body weight was **confirmed**.

**Results:** Three weeks of immobilization stress reduced body weights of males regardless of strain and reduced body weights of Long-Evans females but not of Sprague-Dawley females, replicating and extending past work that examined two weeks of daily stress (e.g., Zylan & Brown, 1996; Faraday et al., 1998). Average stress effects were largest among Long-Evans males (13% of variance) and somewhat smaller for Long-Evans females (9.5% of variance) and Sprague-Dawley males (8% of variance). The presence of a larger effect among Long-Evans vs. Sprague-Dawley males — a within-sex strain difference — is a new finding and probably results from the greater statistical power available in the present experiment. Sprague-Dawley female body weights were statistically unaffected by stress with the exception of a transient stress effect on Stress Day 9.

**Hypothesis 2:** The hypothesis that stress would reduce male feeding regardless of strain and would reduce Long-Evans female feeding, but would have no effect on Sprague-Dawley female feeding was **partially confirmed**.

**Results:** Over three weeks of daily stress, stress significantly reduced food consumption of males, with greater effects in Long-Evans males (10.5% of variance) than in Sprague-Dawley males (7.3% of variance). This finding replicates and extends past reports (Faraday et al., 1998; Zylan & Brown, 1996).

As with body weight findings, the larger effect in Long-Evans males vs. Sprague-Dawley males probably derives from the greater sensitivity of this experiment (i.e., the consequence of greater statistical power).

In addition, as hypothesized, stress did not affect feeding of Sprague-Dawley females, replicating and extending past work (Faraday et al., 1998; Zylan & Brown, 1996). In contrast to hypothesized results, however, stress also did not reduce feeding by Long-Evans females. Possible reasons for this divergence are addressed in the Discussion.

**Hypothesis 3:** The hypothesis that stress would alter locomotion of Sprague-Dawley males early in the Stress Phase, alter locomotion of Sprague-Dawley and Long-Evans females late in the Stress Phase, and would not alter Long-Evans male locomotion was **partially confirmed**.

**Results:** Stress reduced activity (horizontal activity and/or total distance) of Long-Evans males, Sprague-Dawley males, and Sprague-Dawley females on Stress Day 1, with the largest effects in Long-Evans males. Stress also reduced exploration (vertical activity) of Sprague-Dawley females on Stress Day 1. On Stress Day 9, Stress reduced activity and exploration of Long-Evans males only. On Stress Day 19, Stress reduced activity, exploration, and time in the center of the open field for Sprague-Dawley females.

As hypothesized, therefore, stress did alter locomotion of Sprague-Dawley males early in the Stress Phase and of Sprague-Dawley females later in the Stress Phase. In contrast to hypothesized results, however, stress: 1) altered Sprague-Dawley female locomotion at the beginning and at the end of the Stress

Phase; 2) altered Long-Evans male locomotion during the first nine days of the Stress Phase; and 3) did not affect Long-Evans female locomotion at all during the Stress Phase.

**Hypothesis 4:** The hypothesis that stress would increase male Sprague-Dawley acoustic startle amplitude without changes in percent pre-pulse inhibition (PPI), decrease Long-Evans female startle and increase percent PPI, and have no effect on startle or PPI of female Sprague-Dawleys or male Long-Evans was **partially confirmed**.

**Results:** Stress increased startle of male Sprague-Dawleys on every measurement day and generally did not affect percent PPI, as hypothesized. Also as hypothesized, stress had no effect on Long-Evans male startle or PPI. These findings replicate and extend earlier reports (Acri, 1994; Faraday et al., 1999). In contrast to hypothesized results, however, stress increased startle and decreased percent PPI of Sprague-Dawley females and decreased percent PPI of Long-Evans females. These findings may be the consequence of increased statistical power in the present experiment as well as the fact that more sensitive equipment was used than in previous work. These possibilities are addressed in the Discussion.

**Hypothesis 5:** The hypothesis that stress would alter passive avoidance performance for all subgroups was **partially confirmed**.

**Results:** Stress did not alter passive avoidance performance of Sprague-Dawley males or females, or of Long-Evans males, but did impair the performance of Long-Evans females.

**Hypothesis 6:** The hypothesis that stress would alter Morris water maze performance for all subgroups was **partially confirmed**.

**Results:** Stress impaired search strategy efficiency (Trial 1 performance) of Sprague-Dawley females and improved search strategy efficiency of Long-Evans males. Stress did not consistently alter search strategy efficiency of Sprague-Dawley males or Long-Evans females, and did not consistently affect spatial memory performance (Trial 2 performance) for any subgroups. Sex and strain differences in motivation to perform the task may be relevant to these findings and are addressed in the Discussion.

**Hypothesis 7:** The hypothesis that stress would increase HPA axis hormone levels (corticosterone, ACTH, and CRF) for all groups except for Long-Evans females was **partially confirmed**.

**Results:** For animals that were sacrificed on Stress Day 11, Stress increased Sprague-Dawley male and female corticosterone and ACTH levels, and increased Long-Evans male corticosterone levels, as hypothesized. Long-Evans male ACTH also increased as result of Stress but the increase was not statistically significant, probably because of variance and the relatively small  $n$  ( $n = 4$ ). Further, as hypothesized, Stress did not significantly increase Long-Evans female corticosterone or ACTH. Stress decreased CRF of Sprague-Dawley animals — opposite to the hypothesized direction — but increased CRF of Long-Evans animals — consistent with the hypothesis.

For animals that were sacrificed on Stress Day 21, as hypothesized, Stress increased corticosterone and ACTH levels of Sprague-Dawley males and

females, and of Long-Evans males. Stress also increased Long-Evans female corticosterone — in contrast to hypothesized results — but not ACTH — consistent with hypothesized results. In contrast to findings from Stress Day 11, the hypothesized increase in CRF as a result of Stress did not occur for any subgroup after three weeks of stress.

## DISCUSSION

The goal of this doctoral research was to identify potential behavioral and possibly biochemical markers of stress vulnerability in male and female rats of two strains that could be used to predict eventual development of specific stress-related behavioral disorders or diseases in certain subgroups. These markers are conceptualized as correlating with specific underlying vulnerabilities and therefore predicting certain disease states (see Figure 1 in the Introduction). The experiment assessed effects of mild, repeated daily stress on multiple behaviors and biochemical indices within the same subjects to construct a detailed model of possible stress vulnerability markers.

In particular, the specific aims of this research were to: characterize behavioral and biochemical stress responses in a rat model across a range of dependent variables within the same subjects to begin construction of a stress vulnerability model; operationalize potential vulnerability subgroups as male and female rats of two strains; and determine the extent to which differential patterns of behavioral and biochemical stress responses existed among these subgroups. A further goal of the experiment was to characterize patterns of stress responses in terms of their implications for stress vulnerability vs. resilience.

The major findings of the experiment were: 1) the four subgroups (i.e., two strains of male and female rats) manifested behaviors within each behavioral domain that imply differential stress vulnerability (i.e., feeding, body weight, and

activity vs. cognitive performance);<sup>5</sup> 2) the four subgroups manifested relatively consistent behaviors on the measures within each behavioral domain in response to stress, suggesting that underlying stress vulnerabilities may be largely domain-specific; and 3) stressed animals within each of the four subgroups manifested changes in hypothalamo-pituitary-adrenocortical (HPA) axis hormones consistent with a stress response. Therefore, behaviors, rather than hypothalamo-pituitary-adrenocortical axis hormones, most clearly differentiated the rat subgroups in terms of responses to stress. In addition, findings suggest that certain behaviors (i.e., feeding, acoustic startle and prepulse inhibition) may have utility as markers for domain-specific types of stress vulnerability.

The **Results Summary** section below summarizes the findings for each dependent variable. Findings then are discussed in terms of implications for stress vulnerability vs. resilience, relevant methodologic issues are addressed, and specific future studies are outlined. The section that follows addresses limitations of the present experiment. The final section addresses the use of particular behaviors as potential stress vulnerability markers and places the findings in the broader context of the stress literature.

### **Results Summary**

Table 16 below summarizes the findings from behavioral and biochemical

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The fact that effects of stress varied depending on the domain assessed indicates the importance of measuring more than one type of response to understand stress vulnerability in the broadest sense.

data. Arrows indicate the direction of the Stress effect on each measure. The number of arrows indicates the approximate relative magnitude of the effect. Shaded boxes for behavioral measures indicate Stress effects with negative outcomes. The single positive behavioral Stress effect — increased search strategy efficiency by Long-Evans males — is designated with an upward arrow in a non-shaded box. For biochemical measures, specific boxes are not shaded because it is less clear what type of response constitutes a negative outcome.

**Table 16. Summary of Stress effects during the Stress Phase.**

<b>Dependent Measure</b>	<b>Sprague Dawley</b>		<b>Long-Evans</b>	
	<b>Male</b>	<b>Female</b>	<b>Male</b>	<b>Female</b>
Body Weight	↓	↔	↓↓	↓
Food Consumption	↓	↔	↓↓	↔
Locomotion	↓	↔	↓↓	↔
ASR	↑↑	↑	↔	↔
Sensory-gating (ASR w/ PPI)	↓	↓↓	↔	↓↓
Passive avoidance	↔	↔	↔	↓
Water maze: Search strategy	↔	↓	↑	↔
Water maze: Spatial memory	↔	↔	↔	↔
Corticosterone	↑↑↑↑	↑↑↑	↑	↑
ACTH	↑↑↑	↑↑	↑	↔
CRF (Day 11/Day 21)	↓↔	↓↓↔	↑↑↔	↑↔

**Body weight, feeding, and locomotion.** With regard to body weight, feeding, and activity, males were more sensitive to stress than were females,

exhibiting significant reductions on both measures. Among males, Long-Evans males were the most stress-sensitive group, with the largest decreases in body weight, feeding, and activity. Although females were less affected by stress on these measures than were males, Long-Evans females were more sensitive than were Sprague-Dawley females, with stress-induced body weight decreases present through much of the experiment. Sprague-Dawley females were the most stress-resistant of all animals on these measures, with body weight, feeding responses, and activity unaltered by stress.

These results generally replicate and extend past work on stress and feeding in these two strains of male and female rats (e.g., Faraday et al., 1998; Zylan & Brown, 1996), and make clear that the sex-strain subgroups exhibit differential responses to stress on these measures.<sup>6</sup>

With regard to relevance to the human condition, this animal model is consistent with human findings in that sex differences were evident in effects of stress on feeding and, in particular, that stress decreased feeding for males (e.g., Grunberg & Straub, 1992; Grunberg & Klein, 1995; Klein, Faraday, & Grunberg, 1996). These animal findings diverge from the human literature, however, in one important respect — there were no incidences of increased feeding in response to stress. This divergence might highlight an important

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The only divergence from past work (Faraday et al., 1998) was that stress did not reduce feeding by Long-Evans females. Two factors may be relevant to this outcome. First, the present experiment had double the animals per cell as compared to past work ( $n = 20$  vs.  $n = 10$ ), ensuring optimal statistical power. The earlier finding, therefore, may have been the result of sampling variation. Second, it is possible that measurement of behaviors (i.e., locomotion, acoustic startle, passive avoidance, water maze) in addition to feeding behaviors affected feeding behavior. See detailed discussion of this possibility under Limitations.

difference between rat and human behavior and indicate that the rat model does not completely parallel the human condition. However, it also is relevant that animals in the present experiment and in most past work had access only to bland rat chow. This methodologic difference between the animal experiments and the human condition — that humans are free to consume a variety of foodstuffs before, during, and after stress, and the experimental animals were not — also could explain the discrepancy.

Therefore, these findings make clear the need for the next experiment in this series on stress and feeding: non-stressed and stressed adult male and female Sprague-Dawley and Long-Evans rats with access to foods that vary in the major macronutrients (protein, fat, carbohydrate) and the major taste classes (sweet, salty, bland). Control groups with access only to bland rat chow also should be included as comparison points and to clarify the effects of stress on fully adult Long-Evans female feeding when only bland food is available. In addition, because sex and strain differences in stress effects on body weight and feeding appear generally reliable, future experiments should begin the complex process of pinpointing central mechanisms for the differences. Specifically, the actions of CRF, neuropeptide Y (a peptide that regulates carbohydrate consumption), galanin (a peptide that regulates fat consumption), and the sex hormones — substances relevant to feeding, metabolism, and stress, and known to interact at the level of the hypothalamus — need to be examined.

With regard to locomotion findings, several issues emerged. Other investigators have reported that footshock decreased horizontal activity in male

Sprague-Dawleys and that immobilization decreased horizontal activity in male Long-Evans (Lemoine et al., 1990; Trudeau et al., 1990).

In the present experiment, findings generally replicated those of other investigators (Lemoine et al., 1990; Trudeau et al., 1990).<sup>7</sup> Stress decreased activity and exploration variables but not center time. These effects were present in Sprague-Dawley males and females on Stress Day 1 and in Long-Evans males on Stress Days 1 and 9, and were the largest (in terms of proportion of variance explained) in Long-Evans males. In addition, on Stress Day 19 stress decreased activity, exploration, and center time for Sprague-Dawley females. Female Long-Evans locomotion was unaffected by stress throughout the experiment, indicating that this group was the most stress-resistant on this measure.

One interesting aspect of these findings, however, is that increased activity (i.e., exercise) in response to stress, as advocated by Cannon (1933) to prevent destructive consequences of sympathetic nervous system activation, does not appear to be a reflexive, instinctual, hard-wired response in animals. Therefore, it also is likely that humans will not involuntarily engage in exercise in response to stress, and that exercise as a stress management technique to manage negative physiological stress effects requires volition, effort, and

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In pilot work, immobilization stress generally decreased Sprague-Dawley male and female center time (indicating possible increased anxiety), and Long-Evans female horizontal activity, total distance, and vertical activity. Long-Evans male locomotion behaviors were not altered by stress in that experiment. Findings reported here may have diverged from pilot work for the reasons discussed in Footnote 6 regarding Long-Evans female feeding, including issues of sampling variation and statistical power.

commitment. Being aware that exercise may run contrary to one's natural (unhealthy) impulses may be useful information for individuals having difficulty maintaining an exercise program.

An important methodologic consideration also emerged from the effects of locomotion measurement on feeding: the possibility that the two hour locomotion measurement experience was itself a stressor. This possibility is worth exploring for several reasons. First, stressors most relevant to the human experience are psychological — that is, experiences that provoke anxiety, worry, and challenge (and consequent sympathetic nervous system and HPA axis activation) without physical manipulation. Rodent paradigms for psychological stressors are relatively limited and generally involve the conditioning of fear to a painful stimulus. The logistics of maintaining the conditioning (i.e., frequent re-exposure to the painful stimulus) and that fact that physiological changes associated with physical pain also are involved limit the utility of these approaches in some contexts. If the open field experience constitutes a robust psychological stressor, as suggested by the feeding decreases, then this measure may be a useful means of producing stress that is relevant to human concerns, does not involve physical manipulation or pain for the animal, and avoids the complexities of conditioning paradigms.

Locomotion measurement as a stressor could be validated by exposing animals to the open field and measuring corticosterone responses. The feeding data suggest that this experience was more stressful for Long-Evans than for Sprague-Dawleys because Sprague-Dawley feeding stabilized by Stress period

5-7 (there were no compensatory feeding increases after this period) but Long-Evans feeding did not. If so, then this "stressor" might be a useful tool for delineating strain differences in response to psychological stress.

**Cognitive measures. Acoustic startle reflex.** This pre-cognitive, non-volitional measure reveals reactivity to an unexpected stimulus and, when coupled with a pre-pulse, indexes sensory-gating, information-processing, and possibly attention (Swerdlow et al., 1992; Acri, 1994; Acri et al., 1991, 1994, 1995; Grunberg et al., 1994; Faraday et al., 1998, 1999). Increases in startle without a change in percent pre-pulse inhibition (PPI) or decreases in PPI with or without startle increases indicate a net impairment of sensory-gating processes.

In the present experiment, females were more sensitive to stress effects on this measure than were males. Among females, Sprague-Dawley females were the most affected, with stress increasing startle and decreasing percent pre-pulse inhibition (PPI) on three of the four measurement days. For Long-Evans females, stress did not alter startle but decreased percent PPI on three of the four measurement days.

Males were relatively resistant to these stress effects. Stress increased startle of male Sprague-Dawleys on every measurement day but generally did not affect percent PPI, indicating that Sprague-Dawley males maintained consistent sensory-gating. Long-Evans males were the most stress-resistant subgroup, with no stress effects on startle or PPI throughout the experiment.

The findings with male Sprague-Dawleys and male Long-Evans replicate and extend earlier reports (Acri, 1994; Faraday et al., 1999) and indicate that for

males strain is a critical variable in effects of stress on reactivity and sensory-gating. The findings with females do not replicate past work. There are several possibilities that might explain findings with females. The single greatest difference between this experiment and others done in this laboratory is that this experiment involved the use of new equipment (Med Associates Acoustic Startle Test System). The Med Associates system differs in two ways from older equipment (Coulbourn Instruments) used in past experiments (i.e., Acri, 1994; Faraday et al., 1999). First, it is more sensitive and reliable than the Coulbourn equipment in terms of stimulus presentation, response measurement, and data collection. Second, the Med Associates system tests animals in individual chambers (the most widely-used procedure in the literature) while the Coulbourn system tested groups of animals simultaneously within the same chamber. In a study that compared the different testing situations, social parameters of the testing environment (testing alone vs. testing in groups) affected female responses more than male responses (Faraday & Grunberg, 2000). Whether ASR and PPI responses to stress also vary depending on the social context of the testing environment is not known. The unexpected findings with females, however, may be a consequence of this testing difference from earlier experiments.

In addition, startle and PPI responses are well-known as behavioral indices that exhibit substantial inter-subject variability. The large cell size in this experiment ( $n = 20$ ) was intended to provide sufficient statistical power to detect differences despite large expected variance. Findings with females, therefore,

also may have emerged as a consequence of increased statistical power in the present experiment as compared to past work. In any case, findings with females need to be replicated.

It also would be useful to examine stress effects on other, more complex tests of attention in order to develop a more detailed picture of individual differences in attentional effects of stress. Tests such as the latent inhibition paradigm, in which the extent to which animals attend to a tone that previously was paired with shock is measured, could provide this kind of information. In addition, more complete information about attentional processes would be valuable to interpret stress effects on complex cognitive measures (i.e., passive avoidance, water maze) that involve multiple cognitive processes (i.e., attention, cue encoding, cue retrieval).

Human experiments also would make valuable contributions to this question. ASR and PPI can be measured in humans and these responses involve the same underlying circuitry as in rodents and other mammals. To-date, effects of stress on ASR and PPI in humans have not been studied. It would be worthwhile to determine whether the differences observed in rat ASR and PPI responses to stress also are present in different subgroups of people. In addition, because many different paper-and-pencil and computerized tasks exist that measure specific aspects of attentional processing, a detailed picture of stress effects across different attentional domains (i.e., selective attention, focused attention, divided attention) and of individual differences in these effects could be constructed. Further, the extent to which stress-induced changes in

ASR and PPI (reflexive processing) predict changes in other domains could be assessed. If ASR and PPI responses are predictive of other changes and distinguish subgroups of human subjects, then animal experiments could begin the process of pinpointing neurochemical mechanisms for these differences. Information about possible underlying neurochemical differences would be useful to understand human individual differences in stress effects on attentional processes and also might point toward optimal strategies (cognitive, behavioral, pharmacologic) for managing attentional problems in vulnerable individuals.

***Passive avoidance.*** This simple memory task revealed the extent to which animals remembered an aversive experience (foot shock) 24 hrs previously. The only effect of stress was to impair the performance of Long-Evans females — the most stress-sensitive group on this measure. Stress did not alter performance of other subgroups although Long-Evans animals generally performed the task better than did Sprague-Dawleys. Using a different strain (Wistars), others have reported that intense stressors delivered before the training session (i.e., repeated foot shock, swim stress) improved memory performance in males (Kumar & Karanth, 1996; Pare, 1996). Stress-induced memory improvement in males was not observed in the present experiment, perhaps because the immobilization stressor was relatively mild or because of strain differences in stress effects (i.e., Wistars vs. Sprague-Dawleys and Long-Evans). It also is possible that improvement would have been observed if animals had been stressed before training but not before testing, a procedure

that the design of the present experiment did not allow.<sup>8</sup>

As with the ASR and PPI results, these results suggest the need for human experiments to clarify individual differences in stress effects on simple memory and to construct a more detailed model of these effects. If subgroups of normal human subjects also exhibit differential memory responses when exposed to a mild stressor, then animal studies can be used to identify central mechanisms that might explain the differences.

***Morris water maze.*** This complex cognitive task provides information about two types of cognitive performance: search strategy efficiency (Trial 1 performance) and spatial memory (Trial 2 performance). Stress impaired search strategy efficiency of Sprague-Dawley females — the most stress sensitive subgroup on this measure — and improved search strategy efficiency of Long-Evans males. Stress did not consistently alter spatial memory performance of any subgroup although males performed better than did females. The findings on search strategy are new and extend the existing literature, especially because Trial 1 performance is generally ignored as a variable. Because stress effects on spatial memory performance in this measure have not been studied, the fact that stress did not alter this type of performance also adds to the literature. However, interpretation of these findings must be qualified by the fact that males and

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One important methodologic qualification is necessary with regard to passive avoidance results. Animals were trained on Stress Day 4 and tested on Stress Day 5. Animals had been exposed to the stressor for several days, therefore, prior to testing in this measure. It is possible that results may have varied if timing of the measurement during the Stress Phase (i.e., earlier or later) had been different. Therefore, replication of these findings after different periods of stress exposure is important.

females appeared to differ in motivation to do the task.

In theory, animals are motivated to find the platform on both trials because the water is aversive and the platform provides a means of escape. However, on each day a substantial number of Long-Evans females would not remain on the platform at the end of Trial 1 for the required 30 sec. Failure to remain on the platform at the end of Trial 1 rendered performance on Trial 2 uninterpretable because animals did not observe their surroundings while on the platform (a necessary component for spatial memory performance). This pattern was strongest among non-stressed Long-Evans females, with a third or more of that subgroup not sitting on each day. Interestingly, stress increased the likelihood of sitting for Long-Evans females, suggesting that stress increased motivation to do the task.

The experimenter's observation was that non-stressed Long-Evans females were more motivated to escape the water maze entirely than they were to escape the water by sitting on the platform. On every testing day these animals swam repeatedly around the perimeter of the tank — a behavior that was present in all subgroups initially but that disappeared over the first few days as it became clear that the platform was never located near the tank edge. Examination of Trial 1 performance over the five testing days supports this observation, with non-stressed Long-Evans females taking longer and swimming further each day to find the platform on Trial 1. In addition, both Long-Evans and Sprague-Dawley females that did sit on the platform immediately jumped off of it when the experimenter approached the tank and swam toward the experimenter,

presumably because they had learned that the experimenter would remove them from the tank. In contrast, males of either strain would not leave the platform and waited to be picked up off of it. This observation suggests a more generalized sex difference in terms of the aversiveness of the water.

Clearly, interpretation of sex, strain, and stress effects on spatial memory performance depends on methodologic details that might affect motivation. With regard to sex differences in particular, Roof and Stein (1999) used the identical task as in the present experiment (but without a stressor), and reported that male and female Sprague-Dawley rats performed the task equally well. There were two differences in the methodology between that study and this experiment: water temperature and use of the experimenter as a cue.

With regard to water temperature, Roof and Stein (1999) used 20°C water; in the present experiment the water was approximately 30°C. Relatively warm water was used in the present experiment because of reports that water in the 19-20°C range resulted in increased levels of corticosterone (Sandi et al., 1997), consistent with a stress response. Because the present experiment used stress as an independent variable, effort was made to minimize the stressful nature of maze conditions. These results suggest, however, that for females warm water may not be aversive enough to provide sufficient motivation to perform the task.

The other methodologic difference between the present experiment and the Roof and Stein (1999) study was the use of the experimenter as a spatial navigation cue by having the experimenter stand in the same place during every

trial. In an earlier study, in which the experimenter changed position on each trial, males performed better than did females (Roof & Stein, 1999). Roof and Stein (1999) interpreted the disappearance of the sex difference when the experimenter stood in the same place as evidence that females were using the experimenter as a navigation cue and males were not. When the experimenter changed position, therefore, female performance suffered while male performance remained intact.

The experimenter was not used as a cue in the present experiment because pilot work indicated that females swam toward the experimenter rather than searching for the platform. Whether female performance would have improved if the experimenter had been a cue, therefore, is not clear. This issue, as well as the question of water temperature, need to be resolved before firm statements about the nature of stress effects on water maze performance can be made.<sup>9</sup>

These findings also make clear the interpretive difficulties that arise when complex animal behaviors are measured that may be sensitive to procedural differences, when motivational states must be inferred from behaviors, and when the subject cannot be asked directly why he or she used a particular strategy. As with the other cognitive measures, therefore, human experiments would illuminate these issues. In particular, use of a virtual reality water maze has

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As with passive avoidance findings, it is important to note that animals were tested in the water maze partway through the Stress Phase (from Days 12 through 18). These results, therefore, may be specific to situations in which subjects have had many exposures to the stressor.

been reported with human subjects (Astur, Ortiz, & Sutherland, 1998). The use of a virtual reality paradigm eliminates methodologic problems such as water temperature although it may not completely resolve the issue of differential motivations by the genders. Interestingly, Astur et al. (1998) reported over a series of experiments that men outperformed women when the platform was hidden but always located in the same place, even when subjects were told that the platform was always in the same place. The issue of differential motivation, however, was not assessed in this study. It would be fruitful to manipulate task demands (i.e., monetary or other rewards for better performance) in order to more clearly address the question of motivation. In addition, specific cues in the virtual reality environment could be varied (i.e., use of objects vs. people) to determine whether the reported sex difference is stable or, as may be the case with male and female rats, a function of cue type. Further, the effects of stress on performance in this paradigm have not been assessed, but would be a valuable contribution once methodologic issues are resolved, especially if coupled with measurement of stress and sex hormones in saliva.

**HPA Axis hormones.** HPA axis hormones (corticosterone, ACTH, and CRF) were measured to validate the stressor and as possible mechanisms for certain behavioral effects of stress. With regard to corticosterone and ACTH, Sprague-Dawley animals responded more robustly to stress than did Long-Evans, and within each strain males responded more than did females. More specifically, stress increased corticosterone and ACTH of subgroups measured on Stress Day 11 and on Stress Day 21 with the exception of Long-Evans

females, for which Stress increased corticosterone only on Stress Day 21.

With regard to the strain differences in corticosterone and ACTH responses to stress, several questions remain to be answered. For example, it has been argued that effective behavioral coping is reflected by a prompt HPA axis response when confronted with a stressor that is efficiently turned off when the stressor ceases (McEwen et al., 1986; McEwen, 1998). According to this argument, Sprague-Dawleys continued to respond appropriately to repeated stressor presentation and Long-Evans responses may have become inappropriately dampened. On the other hand, failure to habituate biochemically after repeated stressor exposures also can be interpreted as an inappropriate and health-threatening response (McEwen, 1998). By this argument, Long-Evans animals exhibited appropriately muted responses while Sprague-Dawleys exhibited possibly unhealthy failure to habituate. This issue cannot be resolved from the present data because hormones were not measured after each occasion of stressor presentation (i.e., samples were taken only after 11 and 21 days of stress). A future study should sample hormones repeatedly in the two strains to determine whether Sprague-Dawley vs. Long-Evans exhibit different hormonal responses to stress initially or whether these differences emerge over time. It is worth noting that there is little difference between the corticosterone and ACTH responses measured on Stress Day 11 vs. those measured on Stress Day 21, suggesting that animals did not become sensitized to the stressor between days 11 and 21 of stress.

The small differences in corticosterone and ACTH responses between

non-stressed vs. stressed Long-Evans females are consistent with past work (Faraday & Grunberg, unpublished data). The small differences appear to be the result of large within-group variability in hormonal responses in combination with the smaller magnitude responses of Long-Evans in general to stress. Whether Long-Evans females inherently exhibit greater hormonal variability, whether these variations indicate that some animals had habituated to the stressor while others had not, or whether the variability reflects extreme sensitivity to environmental events in some but not all animals is not clear. Within-subject repeated sampling would resolve these questions.

With regard to CRF responses, on Stress Day 11 stress decreased CRF of Sprague-Dawley animals but increased CRF of Long-Evans animals and stress had no effect on CRF levels on Stress Day 21. These results are intriguing because they suggest that by Stress Day 11 CRF responses in stressed Sprague-Dawleys were reduced compared to non-stressed Sprague-Dawleys despite the fact that stressed animals exhibited robust corticosterone and ACTH responses. This interpretation is consistent with Culman, Kopin, and Saavedra (1991) who reported that repeated exposure to the same stressor resulted in reduced CRF release from the median eminence in male Sprague-Dawleys while corticosterone responses remained robust. Culman et al. (1991) interpreted these findings to mean that HPA axis adaptation to repeated stressor presentation occurs at the level of the hypothalamus and allows for intact corticosterone responses. The increased CRF levels in stressed Long-Evans, therefore, would suggest that these animals had not adapted to the stressor by

Stress Day 11. Data from Stress Day 21 are harder to interpret, but the lack of differences between non-stressed and stressed animals at this point may suggest that in later phases of adaptation CRF levels return to normal and that both groups had adapted. As with the corticosterone and ACTH data, these questions would best be answered with repeated sampling from the same subjects over time.

It also is worth noting that Long-Evans females had high CRF levels at both measurement points. Several investigators have found a positive association between CRF levels, anxiety-related behaviors, and anxiety disorders (Dunn & Berridge, 1990; Kalin, 1990; Koob & Britton, 1990; Owens & Nemeroff, 1991; Swerdlow et al., 1986; Britton et al., 1982; Morley & Levine, 1990; De Souza, 1990; Nemeroff et al., 1988; Owens & Nemeroff, 1991; Roy-Byrne et al., 1986; Gold et al., 1986). It is possible that Long-Evans female water maze behaviors (i.e., the apparent preoccupation with escaping the tank rather than finding the platform) were evidence of anxiety and were related to the high CRF levels in this group. Because gold standard behavioral measures of animal anxiety were not included in the present experiment (locomotion center time is not a clear measure of anxiety and did not yield in the present experiment), it is not clear whether this subgroup was more anxious than the other subgroups at baseline and/or in response to stress. Future studies could examine this possibility by using validated anxiety measures (e.g., light-dark box, elevated plus maze) and assessing hormonal responses with and without stress.

In addition, other biochemical processes clearly are relevant to fully

understand subgroup differences in stress behavioral effects. For example, the catecholamines, and in particular the dysregulation of noradrenergic pathways has been implicated in hypertension, congestive heart failure, and depression (Robertson et al., 1991; Goldstein, Lenders, Kaler, & Eisenhofer, 1996).

Therefore, measurement of catecholamines peripherally and of tyrosine hydroxylase (the rate-limiting enzymatic step in catecholamine biosynthesis) activity and messenger RNA (as well as other catecholaminergic enzymes such as dopamine  $\beta$ -hydroxylase) peripherally (in adrenomedullary tissue) and centrally (i.e., in the locus coeruleus) could significantly expand knowledge about underlying mechanisms for observed individual differences in stress behavioral effects. There is some indication that catecholaminergic processes may better differentiate the groups than HPA axis hormones. In particular, Henry et al. (1993) indicated that hypertension developed by Long-Evans males during exposure to chronic social stress appeared to be partly the consequence of increased adrenomedullary catecholamine synthesis. This effect was not present in Sprague-Dawley males, the group that developed mild hypertension.

#### **Limitations of the present experiment.**

Some aspects of the present experiment limit interpretation. These limitations fall into three categories: 1) limitations that are a consequence of the methodologic approach; 2) limitations that flow from the data analytic approach; and 3) limitations that flow from the process of theory development.

**Limitations: Methodologic Approach.** With regard to limitations that are a consequence of the methodologic approach, several issues are important to

consider. In particular, the multivariate approach necessitated the measurement of many behaviors within the same subject. The strength of this approach is the error variance reduction associated with within-subject designs. The potential weakness of the approach is the possibility that the dependent measures *per se* affected one another. In an effort to control for these effects, animals were measured in only one measure on each day. Nevertheless, certain interactions appear to have occurred. Locomotion effects on feeding are the most salient example in the present experiment, with locomotion measurement resulting in decreased feeding by all groups (regardless of stress status) with compensatory increased feeding in the periods following locomotion measurement. It is worth noting, however, that the relative position of the subgroups did not change. That is, stressed males (animals for which stress decreased feeding), continued to exhibit reduced food consumption in comparison to non-stressed males even as feeding of all the groups fluctuated in response to locomotion. These results make clear, however, the need to use separate groups of animals to study feeding and locomotion in the future.

With regard to the other measures, the risk of measure interaction appears to have varied depending on the measure. For example, the consistency of stress effects on body weight and ASR and PPI over many measurement points suggests that these variables were not affected or were minimally affected by other dependent variables. Before passive avoidance training (on Day 4), animals were given a day's rest (Day 3) from all measures to avoid contaminating training with other measures, so it does not appear likely

that this measure was affected by others. During water maze testing, no other variables were measured. The major difficulty with the maze appears to have been sex differences in motivation to do the task, a point that already has been addressed. It is true, however, that passive avoidance and maze results may be specific to the period during the Stress Phase during which they were conducted. That is, each measure took place after several stressor exposures (passive avoidance on Days 4 and 5 of the Stress Phase and water maze on days 12-17 of the Stress Phase). Ideally, other groups could be run as controls to quantify the extent to which measures affect one another. For example, a group could be exposed to 21 days of the stressor, with only body weight and food consumption measured to test the extent to which these variables were affected by the other measures. Or, a group could be exposed to the stressor and only locomotion responses measured, and so on.

In addition, conclusions about the relative importance of behaviors vs. hormonal data in distinguishing subgroups were made based on only two hormone samplings. This conclusion, therefore, is tentative. Future studies in which repeated hormonal sampling that charts the duration as well as the amplitude of responses and also takes into account possible diurnal hormonal effects (effects that may interact with the sex and strain of animal) are necessary to substantiate this conclusion. These studies are particularly important to clarify the potential role of CRF in behavioral responses because this hormone was the most difficult to interpret based on two samplings.

**Limitations: Analytic Approach.** The analytic approach in the present

experiment treated the subgroups as the unit of analysis. However, a great deal of information remains to be gleaned from examination of intra-subgroup variability in stress effects as well as by linking the responses of individuals within and across domains -- in effect, making the individual subject the unit of analysis. These goals can be accomplished statistically with the use of multiple-regression correlations and causal models. In particular, this analytic approach would indicate the extent to which gradations of responses on potential behavioral markers might be useful in further defining the relevance of these markers to possible underlying stress vulnerabilities.

**Limitations: Theory Development.** The goal of this experiment was to identify behavioral responses to stress that might differentiate rat subgroups and serve as potential markers for different types of underlying stress vulnerability. This experiment was intended to provide data for development and refinement of a theoretical model (see Figure 1); it was not conducted under the umbrella of a substantiated theory. In particular, the experiment did not examine vulnerabilities *per se* — only potential markers of vulnerability. Much work remains to be done to complete theory construction, including: 1) the replication of these results; 2) methodologic refinements as discussed; 3) documentation of similar effects in humans; 4) identification of factors that constitute specific vulnerabilities (i.e., the mechanisms that are reflected by the markers as opposed to the markers themselves); and 5) establishment of causal links between vulnerability mechanisms and eventual disease development. These findings, therefore, should be considered in the context of nascent theory

development.

**Findings in the context of the broader stress literature.**

Individuals differ in their behavioral and biologic responses to stress (e.g., Cannon, 1898; Broadhurst, 1960; Mason, 1968a-e; Acri, 1994; Petrides et al., 1994, 1997; Lupien et al., 1997). These individual differences in stress reactivity or sensitivity affect health-relevant behaviors as well as physical and psychological health (e.g., Friedman & Rosenman, 1959; Krantz & Durel, 1983; Suzuki, George, & Meisch, 1988; Lupien et al., 1995; McEwen, 1998). The prevalence of stress-associated behaviors and diseases in modern society — e.g., cardiovascular disease, diabetes, licit and illicit drug abuse, disordered eating — and the fact that stress has been implicated in the etiology of the leading causes of death in the U.S. (i.e., cardiovascular disease, smoking) indicates that stress sensitivity, and the underlying vulnerabilities possibly reflected by different types of stress sensitivity, is not limited to a small subgroup of individuals. Some of the variables that confer relative stress vulnerability vs. resilience, such as gender, have been identified. Gender does not completely account for patterns of stress-associated problematic behaviors and disease, however, indicating that other factors also influence stress susceptibility. A major premise of this dissertation was that these other factors are, at least in part, biologically-based and consequently behaviorally hard-wired, and therefore can be identified using specific behavioral markers.

For example, behavioral markers have been identified that predict certain health-harming behaviors and disease states in humans. In humans the hostile

Type A behavioral pattern and associated excessive sympathetic activation predict which individuals are most at risk for cardiovascular disease. Behavioral as well as biological markers that typify stress-vulnerable subgroups also have been identified in studies using rats. Animals that exhibit the largest behavioral and biologic responses to stressors also are most sensitive to food reward and most likely to self-administer a range of illicit, abused drugs (Dellu et al., 1993, 1996; Piazza et al., 1989, 1990, 1991) — behaviors that are stress-linked and problematic in humans. In addition, normotensive rats manifest different degrees of hypertension when placed in chronically stressful situations. For example, four months of chronic social stress produced mild hypertension (10 mm Hg systolic blood pressure increase) in Sprague-Dawley male rats. In contrast, Long-Evans males exposed to the same stressor exhibited blood pressure increases double those of Sprague-Dawleys and these increases appeared in the first month of stressor exposure — indicating that this subgroup of normotensive animals was especially vulnerable to hypertensive stress effects in response to chronic stress (Henry et al., 1993).

These findings are relevant to the human condition in two ways. First, greater than 90% of hypertension in humans occurs in individuals without underlying pathophysiology, such as kidney disease, and is assumed to be the long-term consequence of repeated excessive sympathetic activation in response to environmental events — stress sensitivity. The fact that chronic stress can produce this disease in “normal” rats without pre-existing pathophysiology — *animals that were not bred for stress vulnerability* —

indicates that stress vulnerability is widely prevalent among rats just as it is among humans. Second, the fact that this human disease is manifested in rats — in which, presumably, individual differences that are the result of psychological and cultural factors have been removed — indicates that stress-sensitive individuals bring specific underlying behavioral and biologic substrates (i.e., specific vulnerability subtypes) to their potentially stressful transactions with the environment and that some components of stress-linked behavioral disorders and disease states are biologically-based and -determined (e.g., Krantz & Durel, 1983; Lerner & Kannel, 1986; Manuck, Kaplan, & Matthews, 1986; Henry et al., 1993; Baum, Gatchel, & Krantz, 1997).

What if behavioral and biologic markers could be identified that predicted vulnerability vs. resilience to specific stress-related behavioral disorders and disease states — *before the disorder or disease was manifested?* The application of this approach to a variety of ills — i.e., diabetes, hypertension, disordered feeding, drug abuse, cognitive impairments, depression, post-traumatic stress disorder — clearly would be valuable in at least two respects. First, at-risk individuals could be identified early and prevention efforts implemented. Second, the behavioral and biologic markers of vulnerability and resilience could be used as a guide for focused animal work on the specific mechanisms by which stress makes certain behavioral problems and disease states more likely, leading to better treatment and prevention strategies in humans. Behavioral markers in particular would be valuable because they can be measured noninvasively, they reflect the integration of myriad neuroendocrine

processes, and many animal and human behaviors are similar.

Findings from this dissertation suggest that certain unconditioned behaviors — in particular, ASR and PPI behaviors and feeding behavior — may be markers of stress vulnerability vs. resilience in subpopulations of rats. It also is possible that these behaviors might predict eventual development of specific stress-related behavioral disorders, cognitive difficulties, or disease states in humans.

For example, stress disrupted sensory-gating (pre-pulse inhibition of the acoustic startle reflex) in female rats of both strains. Stress also impaired performance on complex cognitive tasks in females (simple memory performance in Long-Evans females and search strategy in Sprague-Dawley females). This stress-induced impairment at the non-volitional, pre-cognitive level, therefore, was predictive of impairments on the complex, volitional cognitive tasks in females. Relatively intact sensory-gating in response to stress in males was associated with intact or improved performance on complex cognitive tasks. These results suggest that the extent to which stress affects reflexive information-processing and sensory-gating could be a marker for the extent to which stress will affect more complex cognitive capacities.

If a predictive relationship between ASR and PPI responses to stress and stress effects on complex cognitive performance can be demonstrated in humans, then use of ASR and PPI responses as a behavioral marker of cognitive stress vulnerability might be useful in several contexts. First, in environments such as the military in which intact performance under stress is

critical, using ASR and PPI responses to identify individuals most vulnerable to performance disruption by stress could be useful. If studies indicated that stress-induced sensory-gating impairments predicted attention-specific impairments on other, more complex tasks, then vulnerable individuals could receive training to bolster these weaknesses. This type of screening might be particularly valuable for individuals such as aircraft carrier pilots, for whom a single, simple attentional error on landing approach can result in loss of expensive aircraft and the lives of aircrew and ship's personnel.

It also is relevant that abnormal startle and sensory-gating have been identified across a range of psychological disorders with cognitive disruption as a component, including post-traumatic stress disorder (PTSD) (e.g., Swerdlow et al., 1992). Up to 18% of individuals exposed to a devastating stressor will develop PTSD (Fairley, 1984; Steinglass & Gerrity, 1989; North et al., 1989; Delahanty et al., 1996). Whether perturbations in startle and sensory-gating always were present in this vulnerable subgroup or whether these abnormalities emerged as a consequence of the disease process is not clear. However, screening of individuals likely to be exposed to extreme stressors (i.e., military personnel, rescue workers, police officers, emergency room personnel) and longitudinal follow-up could indicate whether ASR and PPI abnormalities also predict the likelihood of developing severe reactions to stress. If vulnerable individuals do exhibit sensory-gating abnormalities before stressor exposure, then preventive measures could be attempted (i.e., pharmacologic approaches, stress management, cognitive re-structuring).

Changes in feeding in response to stress also might be useful as behavioral markers of stress vulnerability of a different kind. In the present experiment, the largest stress-induced feeding and body weight decreases occurred in Long-Evans males. These animals also have been found to develop marked hypertension in response to chronic social stress as a result of increased catecholaminergic activity (Henry et al., 1993). Because increased sympathetic activity is known to decrease feeding (in part as a result of decreased gastrointestinal motility and increased blood glucose) (e.g., Beaumont, 1833; Cannon, 1898, 1933), decreased feeding in response to mild stress may be a marker for excessive, and ultimately destructive, sympathetic activity that eventually leads to hypertension and other cardiovascular problems.

This possibility could be examined in two ways. First, individuals who have hypertension vs. those who do not could be presented with mild laboratory stressors and feeding responses measured. Second, a prospective study in which feeding responses to stress are used as predictors of hypertension also could be done. If feeding responses do constitute a marker for excessive sympathetic activation and eventual development of hypertension, then individuals could be identified who are vulnerable to these stress effects — perhaps simply by asking the question: "When you are stressed, do you increase, decrease, or not change what you eat?" The utility of this approach is that early identification would allow prevention efforts, such as stress management techniques, to be implemented before disease processes are established. Because hypertension can begin early in life, with disease

detectable in some individuals by age six, accurate identification of vulnerable individuals and intervention ideally would be done in childhood.

With regard to the broader stress literature, stress as an interaction between an organism and the challenges presented to it by its environment has been a unifying theme throughout the development of the concept of stress (e.g., Cannon, 1935; Mason, 1970; Selye, 1976; Baum et al., 1981, 1982, 1997). In particular, the conceptualization of stress used to guide the work presented here is that an organism's "nonbiological" stress responses — emotional, cognitive, psychological — and "biological" stress responses are two manifestations of a mind-body holism. Stress vulnerability vs. resilience, therefore, is a psychobiologic phenomenon in which vulnerability is a single construct that necessarily has biologic, behavioral, and psychological correlates.

This doctoral research began with two touchstones: Walter B. Cannon's description of the innate resilience to internal or external threats conferred on complex organisms by the powerful flexibility of homeostatic systems and Hans Selye's observation that individuals vary inherently in their resilience to stress, in part because of biologically-based factors, such as gender and genotype. The template for the approach presented here, however, in which multiple variables were measured repeatedly within the same subjects to identify markers that might predict stress vulnerability, was adopted from the work of John W. Mason. Mason's 50 years of work demonstrated that measurement of multiple neuroendocrine responses provides a rich window into the psyche. These responses not only are exquisitely sensitive to stressors but also reflect

environmental influences, the organism's developmental and personal history, psychological and emotional states, and unconscious psychological factors such as repression and denial. The complexity of neuroendocrine responses, and the fact that hormones regulate one another as well as multiple target systems in intricate, mutually-dependent networks, has made it difficult to identify hormonal predictors of stress-related disease vulnerability in individuals who are currently healthy.

The goal of the work presented here was to apply Mason's multivariate approach to behavioral variables in combination with selected endocrine variables (i.e., HPA axis hormones). Behaviors were the primary focus of this work because behaviors represent the observable, measurable output that is the consequence of multiple interacting neuroendocrine factors as well as other biological variables. Behaviors, therefore, arise out of the integration of those myriad influences. In addition, because behaviors are multiply neuroendocrine-dependent, it also is likely that they are less sensitive to transient internal or external environmental changes and provide a more stable type of marker that might readily identify vulnerable subgroups.

Findings from the present experiment suggest that stress vulnerability in different groups of organisms may be manifested by markers in specific behavioral domains. These vulnerabilities appear to be different for male vs. female rats and to differ in magnitude for rat strain subgroups within each sex. Importantly, these sex and strain differences in an animal model — in which the psychological factors that might influence responses, such as appraisal and

coping strategy choices, have been removed — suggest that underlying biological differences (including neuroendocrine differences) constitute these different vulnerabilities. If these results generalize to humans, then similar markers in humans also may correlate with specific, underlying vulnerabilities and may be predictive of certain human stress-related behavioral disorders and eventual disease states. Then, the underlying biology of different types of vulnerability can be ascertained and treatment and prevention tailored to specific vulnerability subtypes.

## **APPENDIX A: TABLES**

**Table 17: Results of ANOVAs on body weight from last day of Baseline Phase.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Strain	50.64 (1,159)	p < 0.001
	Sex	1526.13 (1,159)	p < 0.001
	Stress	0.08 (1,159)	n.s.
	Strain X Sex	55.86 (1,159)	p < 0.001
	Strain X Stress	0.03 (1,159)	n.s.
	Sex X Stress	0.02 (1,159)	n.s.
	Strain X Sex X Stress	0.05 (1,159)	n.s.
SD Males	Stress	0.00 (1,40)	n.s.
SD Females	Stress	0.51 (1,43)	n.s.
LE Males	Stress	0.01 (1,38)	n.s.
LE Females	Stress	0.00 (1,38)	n.s.

**Table 18: Results of repeated-measures ANOVAs on body weights through Stress Day 11.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Time	1117.37 (5,795)	p < 0.001
	Time X Strain	5.90 (5,795)	p < 0.001
	Time X Sex	178.80 (5,795)	p < 0.001
	Time X Stress	9.49 (5,795)	p < 0.001
	Time X Strain X Sex	8.25 (5,795)	p < 0.001
	Time X Strain X Stress	1.82 (5,795)	n.s.
	Time X Sex X Stress	0.53 (5,795)	n.s.
	Time X Strain X Sex X Stress	0.52 (5,795)	n.s.
Males	Time	856.85 (5,390)	p < 0.001
	Time X Strain	8.55 (5,390)	p < 0.001
	Time X Stress	5.50 (5,390)	p < 0.001
	Time X Strain X Stress	1.31 (5,390)	n.s.
Females	Time	273.66 (5,405)	p < 0.001
	Time X Strain	4.36 (5,405)	p = 0.001
	Time X Stress	4.04 (5,405)	p = 0.001
	Time X Strain X Stress	0.89 (5,405)	n.s.
SD Males	Time	453.75 (5,200)	p < 0.001
	Time X Stress	1.33 (5,200)	n.s.
SD Females	Time	148.22 (5,215)	p < 0.001
	Time X Stress	1.39 (5,215)	n.s.
LE Males	Time	412.84 (5,190)	p < 0.001
	Time X Stress	5.75 (5,190)	p < 0.001
LE Females	Time	128.25 (5,190)	p < 0.001
	Time X Stress	3.00 (5,190)	p = 0.012

**Table 19: Results of ANOVAs on body weights averaged over Stress Days 1 through 11.**

Group Tested	Effect	F value (d.f.)	p value
All animals	Strain	59.70 (1,159)	p < 0.001
	Sex	2770.44 (1,159)	p < 0.001
	Stress	8.90 (1,159)	p = 0.003
	Strain X Sex	42.73 (1,159)	p < 0.001
	Strain X Stress	0.07 (1,159)	n.s.
	Stress X Sex	0.11 (1,159)	n.s.
	Strain X Sex X Stress	0.05 (1,159)	n.s.
Males	Strain	64.66 (1,78)	p < 0.001
	Stress	3.53 (1,78)	p = 0.064
	Strain X Stress	0.00 (1,78)	n.s.
Females	Strain	1.53 (1,81)	n.s.
	Stress	7.60 (1,81)	p = 0.007
	Strain X Stress	0.25 (1,81)	n.s.
SD Males	Stress	2.04 (1,40)	n.s.
SD Females	Stress	2.90 (1,43)	n.s.
LE Males	Stress	1.54 (1,38)	n.s.
LE Females	Stress	4.65 (1,38)	p = 0.037

**Table 20: Results of repeated-measures ANOVAs on body weights through Stress Day 21.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Time	1255.06 (11,1397)	p < 0.001
	Time X Strain	9.28 (11,1397)	p < 0.001
	Time X Sex	161.24 (11,1397)	p < 0.001
	Time X Stress	9.02 (11,1397)	p < 0.001
	Time X Strain X Sex	5.06 (11,1397)	p < 0.001
	Time X Strain X Stress	0.72 (11,1397)	n.s.
	Time X Sex X Stress	2.38 (11,1397)	p = 0.006
	Time X Strain X Sex X Stress	0.35 (11,1397)	n.s.
Males	Time	770.11 (10,620)	p < 0.001
	Time X Strain	3.03 (10,620)	p = 0.001
	Time X Stress	6.36 (10,620)	p < 0.001
	Time X Strain X Stress	0.60 (10,620)	n.s.
Females	Time	430.77 (10,650)	p < 0.001
	Time X Strain	21.08 (10,650)	p < 0.001
	Time X Stress	1.55 (10,650)	n.s.
	Time X Strain X Stress	0.71 (10,650)	n.s.
SD Males	Time	431.50 (11,352)	p < 0.001
	Time X Stress	3.28 (11,352)	p < 0.001
SD Females	Time	190.16 (11,385)	p < 0.001
	Time X Stress	2.42 (11,385)	p = 0.006
LE Males	Time	345.76 (11,330)	p < 0.001
	Time X Stress	3.68 (11,330)	p < 0.001
LE Females	Time	289.71 (11,330)	p < 0.001
	Time X Stress	1.22 (11,330)	n.s.

**Table 21: Results of ANOVAs on body weights averaged over Stress Days 1 through 21.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Strain	50.06 (1,127)	p < 0.001
	Sex	2030.93 (1,127)	p < 0.001
	Stress	12.55 (1,127)	p = 0.001
	Strain X Sex	16.90 (1,127)	p < 0.001
	Strain X Stress	0.66 (1,127)	n.s.
	Stress X Sex	1.56 (1,127)	n.s.
	Strain X Sex X Stress	0.03 (1,127)	n.s.
Males	Strain	40.64 (1,62)	p < 0.001
	Stress	7.45 (1,62)	p = 0.008
	Strain X Stress	0.32 (1,62)	n.s.
Females	Strain	8.74 (1,62)	p = 0.004
	Stress	5.24 (1,62)	p = 0.025
	Strain X Stress	0.40 (1,62)	n.s.
SD Males	Stress	2.04 (1,32)	n.s.
SD Females	Stress	1.85 (1,35)	n.s.
LE Males	Stress	4.97 (1,30)	p = 0.033
LE Females	Stress	3.22 (1,30)	p = 0.083

**Table 22. Results of ANOVAs on body weights on each Stress day when all animals were considered together.**

<b>Day</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
Stress Day 1	Strain	74.35 (1,159)	p < 0.001
	Sex	2047.23 (1,159)	p < 0.001
	Stress	1.66 (1,159)	n.s.
	Strain X Sex	74.30 (1,159)	p < 0.001
	Strain X Stress	0.35 (1,159)	n.s.
	Sex X Stress	0.00 (1,159)	n.s.
	Strain X Sex X Stress	0.24 (1,159)	n.s.
Stress Day 3	Strain	65.93 (1,159)	p < 0.001
	Sex	2024.13 (1,159)	p < 0.001
	Stress	4.28 (1,159)	p = 0.04
	Strain X Sex	50.65 (1,159)	p < 0.001
	Strain X Stress	0.11 (1,159)	n.s.
	Sex X Stress	0.11 (1,159)	n.s.
	Strain X Sex X Stress	0.03 (1,159)	n.s.
Stress Day 5	Strain	42.97 (1,159)	p < 0.001
	Sex	2313.19 (1,159)	p < 0.001
	Stress	4.43 (1,159)	p = 0.037
	Strain X Sex	45.09 (1,159)	p < 0.001
	Strain X Stress	0.04 (1,159)	n.s.
	Sex X Stress	0.04 (1,159)	n.s.
	Strain X Sex X Stress	0.02 (1,159)	n.s.
Stress Day 7	Strain	29.03 (1,159)	p < 0.001
	Sex	2684.60 (1,159)	p < 0.001
	Stress	6.12 (1,159)	p = 0.015
	Strain X Sex	19.31 (1,159)	p < 0.001
	Strain X Stress	0.15 (1,159)	n.s.
	Sex X Stress	0.00 (1,159)	n.s.
	Strain X Sex X Stress	0.46 (1,159)	n.s.
Stress Day 9	Strain	53.39 (1,159)	p < 0.001
	Sex	2495.32 (1,159)	p < 0.001
	Stress	15.88 (1,159)	p < 0.001
	Strain X Sex	30.41 (1,159)	p < 0.001
	Strain X Stress	0.03 (1,159)	n.s.
	Sex X Stress	0.29 (1,159)	n.s.
	Strain X Sex X Stress	0.08 (1,159)	n.s.
Stress Day 11	Strain	43.90 (1,159)	p < 0.001
	Sex	2359.77 (1,159)	p < 0.001

	Stress	16.17 (1,159)	p < 0.001
	Strain X Sex	17.27 (1,159)	p < 0.001
	Strain X Stress	1.18 (1,159)	n.s.
	Sex X Stress	0.48 (1,159)	n.s.
	Strain X Sex X Stress	0.02 (1,159)	n.s.
Stress Day 13	Strain	41.31 (1,127)	p < 0.001
	Sex	1712.73 (1,127)	p < 0.001
	Stress	14.20 (1,127)	p < 0.001
	Strain X Sex	7.56 (1,127)	p = 0.007
	Strain X Stress	0.83 (1,127)	n.s.
	Sex X Stress	1.45 (1,127)	n.s.
	Strain X Sex X Stress	0.20 (1,127)	n.s.
Stress Day 15	Strain	46.80 (1,127)	p < 0.001
	Sex	1633.73 (1,127)	p < 0.001
	Stress	11.43 (1,127)	p = 0.001
	Strain X Sex	7.55 (1,127)	p = 0.007
	Strain X Stress	1.23 (1,127)	n.s.
	Sex X Stress	2.50 (1,127)	n.s.
	Strain X Sex X Stress	0.13 (1,127)	n.s.
Stress Day 17	Strain	39.86 (1,127)	p < 0.001
	Sex	1307.95 (1,127)	p < 0.001
	Stress	10.14 (1,127)	p = 0.002
	Strain X Sex	5.29 (1,127)	p = 0.023
	Strain X Stress	0.156 (1,127)	n.s.
	Sex X Stress	1.66 (1,127)	n.s.
	Strain X Sex X Stress	0.01 (1,127)	n.s.
Stress Day 19	Strain	52.93 (1,127)	p < 0.001
	Sex	1475.58 (1,127)	p < 0.001
	Stress	18.27 (1,127)	p < 0.001
	Strain X Sex	5.41 (1,127)	p = 0.022
	Strain X Stress	0.511 (1,127)	n.s.
	Sex X Stress	3.47 (1,127)	p = 0.065.
	Strain X Sex X Stress	0.02 (1,127)	n.s.
Stress Day 21	Strain	35.72 (1,127)	p < 0.001
	Sex	1294.26 (1,127)	p < 0.001
	Stress	10.84 (1,127)	p = 0.001
	Strain X Sex	3.91 (1,127)	p = 0.050
	Strain X Stress	0.05 (1,127)	n.s.
	Sex X Stress	3.03 (1,127)	p = 0.084
	Strain X Sex X Stress	0.05 (1,127)	n.s.

**Table 23: Results of ANOVAs on body weights through Stress Day 21 when males and females were analyzed separately.**

<b>Males</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
<b>Stress Day 1</b>	Strain	94.22 (1,78)	p < 0.001
	Stress	0.51 (1,78)	n.s.
	Strain X Stress	0.37 (1,78)	n.s.
<b>Stress Day 3</b>	Strain	71.34 (1,78)	p < 0.001
	Stress	1.76 (1,78)	n.s.
	Strain X Stress	0.08 (1,78)	n.s.
<b>Stress Day 5</b>	Strain	56.03 (1,78)	p < 0.001
	Stress	1.72 (1,78)	n.s.
	Strain X Stress	0.05 (1,78)	n.s.
<b>Stress Day 7</b>	Strain	33.65 (1,78)	p < 0.001
	Stress	2.1 (1,78)	n.s.
	Strain X Stress	0.03 (1,78)	n.s.
<b>Stress Day 9</b>	Strain	54.05 (1,78)	p < 0.001
	Stress	6.73 (1,78)	p = 0.01
	Strain X Stress	0.00 (1,78)	n.s.
<b>Stress Day 11</b>	Strain	39.94 (1,78)	p < 0.001
	Stress	7.62 (1,78)	p = 0.007
	Strain X Stress	0.51 (1,78)	n.s.
<b>Stress Day 13</b>	Strain	28.28 (1,62)	p < 0.001
	Stress	8.30 (1,62)	p = 0.005
	Strain X Stress	0.62 (1,62)	n.s.
<b>Stress Day 15</b>	Strain	30.03 (1,62)	p < 0.001
	Stress	8.04 (1,62)	p = 0.006
	Strain X Stress	0.70 (1,62)	n.s.
<b>Stress Day 17</b>	Strain	24.84 (1,62)	p < 0.001
	Stress	6.70 (1,62)	p = 0.012
	Strain X Stress	0.09 (1,62)	n.s.
<b>Stress Day 19</b>	Strain	30.52 (1,62)	p < 0.001
	Stress	12.47 (1,62)	p = 0.001
	Strain X Stress	0.24 (1,62)	n.s.
<b>Stress Day 21</b>	Strain	21.03 (1,62)	p < 0.001
	Stress	8.42 (1,62)	p = 0.005
	Strain X Stress	0.00 (1,62)	n.s.

**Table 23 (cont):** Results of ANOVAs on body weights through Stress Day 21 when males and females were analyzed separately.

<b>Females</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
<b>Stress Day 1</b>	Strain	0.00 (1,81)	n.s.
	Stress	1.86 (1,81)	n.s.
	Strain X Stress	0.01 (1,81)	n.s.
<b>Stress Day 3</b>	Strain	1.22 (1,81)	n.s.
	Stress	3.68 (1,81)	p = 0.059
	Strain X Stress	0.03 (1,81)	n.s.
<b>Stress Day 5</b>	Strain	0.03 (1,81)	n.s.
	Stress	3.83 (1,81)	p = 0.045
	Strain X Stress	0.00 (1,81)	n.s.
<b>Stress Day 7</b>	Strain	0.82 (1,81)	n.s.
	Stress	5.16 (1,81)	p = 0.026
	Strain X Stress	0.95 (1,81)	n.s.
<b>Stress Day 9</b>	Strain	3.13 (1,81)	p = 0.08
	Stress	11.57 (1,81)	p = 0.001
	Strain X Stress	0.20 (1,81)	n.s.
<b>Stress Day 11</b>	Strain	5.31 (1,81)	p = 0.024
	Stress	9.67 (1,81)	p = 0.003
	Strain X Stress	0.79 (1,81)	n.s.
<b>Stress Day 13</b>	Strain	12.32 (1,65)	p = 0.001
	Stress	5.99 (1,65)	p = 0.017
	Strain X Stress	0.19 (1,65)	n.s.
<b>Stress Day 15</b>	Strain	16.41 (1,65)	p < 0.001
	Stress	3.17 (1,65)	p = 0.080
	Strain X Stress	0.55 (1,65)	n.s.
<b>Stress Day 17</b>	Strain	14.78 (1,65)	p < 0.001
	Stress	3.30 (1,65)	p = 0.074
	Strain X Stress	0.07 (1,65)	n.s.
<b>Stress Day 19</b>	Strain	23.14 (1,65)	p < 0.001
	Stress	5.49 (1,65)	p = 0.022
	Strain X Stress	0.31 (1,65)	n.s.
<b>Stress Day 21</b>	Strain	14.94 (1,65)	p < 0.001
	Stress	2.25 (1,65)	n.s.
	Strain X Stress	0.19 (1,65)	n.s.

**Table 24: Results of ANOVAs on body weights through Stress Day 21 when subgroups were analyzed separately.**

<b>SD Males</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
Stress Day 1	Stress	1.31 (1,40)	n.s.
Stress Day 3	Stress	0.58 (1,40)	n.s.
Stress Day 5	Stress	1.47 (1,40)	n.s.
Stress Day 7	Stress	1.35 (1,40)	n.s.
Stress Day 9	Stress	3.74 (1,40)	p = 0.06
Stress Day 11	Stress	2.36 (1,40)	n.s.
Stress Day 13	Stress	2.54 (1,32)	n.s.
Stress Day 15	Stress	2.16 (1,32)	n.s.
Stress Day 17	Stress	2.72 (1,32)	n.s.
Stress Day 19	Stress	4.45 (1,32)	p = 0.042
Stress Day 21	Stress	4.35 (1,32)	p = 0.045
<b>SD Females</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
Stress Day 1	Stress	1.51 (1,43)	n.s.
Stress Day 3	Stress	1.63 (1,43)	n.s.
Stress Day 5	Stress	2.45 (1,43)	n.s.
Stress Day 7	Stress	0.95 (1,43)	n.s.
Stress Day 9	Stress	5.48 (1,43)	p = 0.024
Stress Day 11	Stress	2.73 (1,43)	n.s.
Stress Day 13	Stress	2.85 (1,35)	n.s.
Stress Day 15	Stress	0.68 (1,35)	n.s.
Stress Day 17	Stress	1.30 (1,35)	n.s.
Stress Day 19	Stress	2.06 (1,35)	n.s.
Stress Day 21	Stress	2.42 (1,35)	n.s.
<b>LE Males</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
Stress Day 1	Stress	0.00 (1,38)	n.s.
Stress Day 3	Stress	1.22 (1,38)	n.s.
Stress Day 5	Stress	0.49 (1,38)	n.s.
Stress Day 7	Stress	0.79 (1,38)	n.s.
Stress Day 9	Stress	3.02 (1,38)	p = 0.090
Stress Day 11	Stress	5.39 (1,38)	p = 0.026
Stress Day 13	Stress	5.84 (1,30)	p = 0.022
Stress Day 15	Stress	6.23 (1,30)	p = 0.018

Stress Day 17	Stress	4.03 (1,30)	p = 0.054
Stress Day 19	Stress	8.46 (1,30)	p = 0.007
Stress Day 21	Stress	4.08 (1,30)	p = 0.052
LE Females	Effect	F value (d.f.)	p value
Stress Day 1	Stress	0.59 (1,38)	n.s.
Stress Day 3	Stress	2.05 (1,38)	n.s.
Stress Day 5	Stress	1.51 (1,38)	n.s.
Stress Day 7	Stress	4.67 (1,38)	p = 0.037
Stress Day 9	Stress	5.96 (1,38)	p = 0.019
Stress Day 11	Stress	7.24 (1,38)	p = 0.011
Stress Day 13	Stress	3.05 (1,30)	p = 0.091
Stress Day 15	Stress	2.54 (1,30)	n.s.
Stress Day 17	Stress	2.00 (1,30)	n.s.
Stress Day 19	Stress	3.28 (1,30)	p = 0.080
Stress Day 21	Stress	0.44 (1,30)	n.s.

**Table 25: Results of ANOVAs on food consumption from last two days of Baseline Phase.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Strain	7.87 (1,159)	p = 0.006
	Sex	364.33 (1,159)	p < 0.001
	Stress	0.26 (1,159)	n.s.
	Strain X Sex	8.61 (1,159)	p = 0.004
	Strain X Stress	0.71 (1,159)	n.s.
	Sex X Stress	0.13 (1,159)	n.s.
	Strain X Sex X Stress	2.36 (1,159)	n.s.
SD Males	Stress	0.54 (1,40)	n.s.
SD Females	Stress	0.22 (1,43)	n.s.
LE Males	Stress	2.37 (1,38)	n.s.
LE Females	Stress	0.07 (1,38)	n.s.

**Table 26: Results of repeated-measures ANOVAs on food consumption through Stress Day 11.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Time	137.76 (4,636)	p < 0.001
	Time X Strain	46.33 (4,636)	p < 0.001
	Time X Sex	3.65 (4,636)	p = 0.006
	Time X Stress	3.85 (4,636)	p = 0.004
	Time X Strain X Sex	4.90 (4,636)	p = 0.001
	Time X Strain X Stress	1.49 (4,636)	n.s.
	Time X Sex X Stress	0.98 (4,636)	n.s.
	Time X Strain X Sex X Stress	0.99 (4,636)	n.s.
Males	Time	73.55 (4,312)	p < 0.001
	Time X Strain	30.09 (4,312)	p < 0.001
	Time X Stress	2.52 (4,312)	p = 0.041
	Time X Strain X Stress	1.58 (4,312)	n.s.
Females	Time	64.54 (4,324)	p < 0.001
	Time X Strain	17.72 (4,324)	p < 0.001
	Time X Stress	2.19 (4,324)	p = 0.070
	Time X Strain X Stress	0.67 (4,324)	n.s.
SD Males	Time	22.99 (4,160)	p < 0.001
	Time X Stress	0.24 (4,160)	n.s.
SD Females	Time	13.82 (4,172)	p < 0.001
	Time X Stress	0.47 (4,172)	n.s.
LE Males	Time	109.26 (4,152)	p < 0.001
	Time X Stress	5.60 (4,152)	p < 0.001
LE Females	Time	84.19 (4,152)	p < 0.001
	Time X Stress	2.94 (4,152)	p = 0.022

**Table 27: Results of ANOVAs on food consumption averaged over Stress Days 1 through 11.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Strain	31.78 (1,159)	p < 0.001
	Sex	652.21 (1,159)	p < 0.001
	Stress	2.95 (1,159)	p = 0.088
	Strain X Sex	0.15 (1,159)	n.s.
	Strain X Stress	0.18 (1,159)	n.s.
	Stress X Sex	2.29 (1,159)	n.s.
	Strain X Sex X Stress	0.19 (1,159)	n.s.
Males	Strain	11.60 (1,78)	p = 0.001
	Stress	3.65 (1,78)	p = 0.060
	Strain X Stress	0.03 (1,78)	n.s.
Females	Strain	25.50 (1,81)	p < 0.001
	Stress	1.60 (1,81)	n.s.
	Strain X Stress	0.27 (1,81)	n.s.
SD Males	Stress	1.47 (1,40)	n.s.
SD Females	Stress	0.32 (1,43)	n.s.
LE Males	Stress	2.23 (1,38)	n.s.
LE Females	Stress	0.04 (1,38)	n.s.

**Table 28: Results of repeated-measures ANOVAs on food consumption through Stress Day 21.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Time	55.25 (9,1143)	p < 0.001
	Time X Strain	27.57 (9,1143)	p < 0.001
	Time X Sex	1.45 (9,1143)	n.s.
	Time X Stress	2.67 (9,1143)	p = 0.005
	Time X Strain X Sex	2.45 (9,1143)	p = 0.009
	Time X Strain X Stress	1.15 (9,1143)	n.s.
	Time X Sex X Stress	1.02 (9,1143)	n.s.
	Time X Strain X Sex X Stress	0.81 (9,1143)	n.s.
Males	Time	28.09 (9,558)	p < 0.001
	Time X Strain	14.18 (9,558)	p < 0.001
	Time X Stress	1.71 (9,558)	p = 0.084
	Time X Strain X Stress	1.38 (9,558)	n.s.
Females	Time	28.18 (9,585)	p < 0.001
	Time X Strain	15.98 (9,585)	p < 0.001
	Time X Stress	2.00 (9,585)	p = 0.037
	Time X Strain X Stress	0.34 (9,585)	n.s.
SD Males	Time	13.13 (9,288)	p < 0.001
	Time X Stress	0.72 (9,288)	n.s.
SD Females	Time	9.46 (9,315)	p < 0.001
	Time X Stress	1.02 (9,315)	n.s.
LE Males	Time	31.51 (9,270)	p < 0.001
	Time X Stress	2.64 (9,270)	p = 0.007
LE Females	Time	32.41 (9,270)	p < 0.001
	Time X Stress	1.29 (9,270)	n.s.

**Table 29: Results of ANOVAs on food consumption averaged over Stress Days 1 through 21.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Strain	50.44 (1,127)	p < 0.001
	Sex	468.22 (1,127)	p < 0.001
	Stress	5.02 (1,127)	p = 0.027
	Strain X Sex	0.54 (1,127)	n.s.
	Strain X Stress	0.12 (1,127)	n.s.
	Stress X Sex	4.85 (1,127)	p = 0.029
	Strain X Sex X Stress	0.45 (1,159)	n.s.
Males	Strain	13.13 (1,62)	p = 0.001
	Stress	6.39 (1,62)	p = 0.014
	Strain X Stress	0.33 (1,62)	n.s.
Females	Strain	61.60 (1,65)	p < 0.001
	Stress	0.00 (1,65)	n.s.
	Strain X Stress	1.05 (1,65)	n.s.
SD Males	Stress	3.02 (1,32)	p = 0.092
SD Females	Stress	1.05 (1,35)	n.s.
LE Males	Stress	3.35 (1,30)	p = 0.077
LE Females	Stress	0.31 (1,30)	n.s.

**Table 30.** Results of ANOVAs on food consumption during each Stress period when all animals were considered together.

Day	Effect	F value (d.f.)	p value
Stress Days 1-3	Strain	1.40 (1,159)	n.s.
	Sex	161.50 (1,159)	p < 0.001
	Stress	0.03 (1,159)	n.s.
	Strain X Sex	1.60 (1,159)	n.s.
	Strain X Stress	0.99 (1,159)	n.s.
	Sex X Stress	0.25 (1,159)	n.s.
	Strain X Sex X Stress	0.19 (1,159)	n.s.
Stress Days 3-5	Strain	12.03 (1,159)	p = 0.001
	Sex	346.55 (1,159)	p < 0.001
	Stress	0.67 (1,159)	n.s.
	Strain X Sex	0.70 (1,159)	n.s.
	Strain X Stress	1.71 (1,159)	n.s.
	Sex X Stress	0.89 (1,159)	n.s.
	Strain X Sex X Stress	0.13 (1,159)	n.s.
Stress Days 5-7	Strain	0.60 (1,159)	n.s.
	Sex	301.63 (1,159)	p < 0.001
	Stress	12.63 (1,159)	p = 0.001
	Strain X Sex	2.95 (1,159)	p = 0.088
	Strain X Stress	0.55 (1,159)	n.s.
	Sex X Stress	0.71 (1,159)	n.s.
	Strain X Sex X Stress	0.06 (1,159)	n.s.
Stress Days 7-9	Strain	175.25 (1,159)	p < 0.001
	Sex	279.16 (1,159)	p < 0.001
	Stress	4.30 (1,159)	p = 0.040
	Strain X Sex	6.35 (1,159)	p = 0.013
	Strain X Stress	0.09 (1,159)	n.s.
	Sex X Stress	5.41 (1,159)	p = 0.021
	Strain X Sex X Stress	2.59 (1,159)	n.s.
Stress Days 9-11	Strain	0.262 (1,159)	n.s.
	Sex	311.48 (1,159)	p < 0.001
	Stress	1.39 (1,159)	n.s.
	Strain X Sex	3.42 (1,159)	p = 0.066
	Strain X Stress	1.05 (1,159)	n.s.
	Sex X Stress	0.40 (1,159)	n.s.
	Strain X Sex X Stress	0.15 (1,159)	n.s.

Stress Days 11-13	Strain	95.43 (1,127)	p < 0.001
	Sex	155.63 (1,127)	p < 0.001
	Stress	4.66 (1,127)	p = 0.033
	Strain X Sex	0.00 (1,127)	n.s.
	Strain X Stress	0.83 (1,127)	n.s.
	Sex X Stress	1.54 (1,127)	n.s.
	Strain X Sex X Stress	0.00 (1,127)	n.s.
Stress Days 13-15	Strain	42.53 (1,127)	p < 0.001
	Sex	229.37 (1,127)	p < 0.001
	Stress	1.98 (1,127)	n.s.
	Strain X Sex	4.48 (1,127)	p = 0.036
	Strain X Stress	0.76 (1,127)	n.s.
	Sex X Stress	6.88 (1,127)	p = 0.010
	Strain X Sex X Stress	0.63 (1,127)	n.s.
Stress Days 15-17	Strain	35.60 (1,127)	p < 0.001
	Sex	240.78 (1,127)	p < 0.001
	Stress	3.31 (1,127)	p = 0.071
	Strain X Sex	5.75 (1,127)	p = 0.018
	Strain X Stress	1.74 (1,127)	n.s.
	Sex X Stress	8.72 (1,127)	p = 0.004
	Strain X Sex X Stress	0.14 (1,127)	n.s.
Stress Days 17-19	Strain	38.62 (1,127)	p < 0.001
	Sex	152.13 (1,127)	p < 0.001
	Stress	11.74 (1,127)	p = 0.001
	Strain X Sex	0.43 (1,127)	n.s.
	Strain X Stress	0.13 (1,127)	n.s.
	Sex X Stress	1.11 (1,127)	n.s.
	Strain X Sex X Stress	0.96 (1,127)	n.s.
Stress Days 19-21	Strain	2.81 (1,127)	p = 0.096
	Sex	154.74 (1,127)	p < 0.001
	Stress	0.59 (1,127)	n.s.
	Strain X Sex	0.01 (1,127)	n.s.
	Strain X Stress	1.10 (1,127)	n.s.
	Sex X Stress	2.55 (1,127)	n.s.
	Strain X Sex X Stress	0.53 (1,127)	n.s.

**Table 31: Results of ANOVAs on food consumption during each stress period when males and females were analyzed separately.**

<b>Males</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
<b>Stress Days 1-3</b>	Strain	2.02 (1,78)	n.s.
	Stress	0.00 (1,78)	n.s.
	Strain X Stress	0.70 (1,78)	n.s.
<b>Stress Days 3-5</b>	Strain	3.69 (1,78)	p = 0.059
	Stress	0.01 (1,78)	n.s.
	Strain X Stress	0.99 (1,78)	n.s.
<b>Stress Days 5-7</b>	Strain	2.36 (1,78)	n.s.
	Stress	7.35 (1,78)	p = 0.008
	Strain X Stress	0.37 (1,78)	n.s.
<b>Stress Days 7-9</b>	Strain	111.72 (1,78)	p < 0.001
	Stress	8.70 (1,78)	p = 0.004
	Strain X Stress	1.65 (1,78)	n.s.
<b>Stress Days 9-11</b>	Strain	0.68 (1,78)	n.s.
	Stress	1.25 (1,78)	n.s.
	Strain X Stress	0.76 (1,78)	n.s.
<b>Stress Days 11-13</b>	Strain	36.76 (1,62)	p < 0.001
	Stress	4.43 (1,62)	p = 0.039
	Strain X Stress	0.35 (1,62)	n.s.
<b>Stress Days 13-15</b>	Strain	7.32 (1,62)	p = 0.009
	Stress	6.13 (1,62)	p = 0.016
	Strain X Stress	1.05 (1,62)	n.s.
<b>Stress Days 15-17</b>	Strain	4.71 (1,62)	p = 0.034
	Stress	8.43 (1,62)	p = 0.005
	Strain X Stress	0.32 (1,62)	n.s.
<b>Stress Days 17-19</b>	Strain	11.94 (1,62)	p = 0.001
	Stress	7.77 (1,62)	p = 0.007
	Strain X Stress	0.71 (1,62)	n.s.
<b>Stress Days 19-21</b>	Strain	0.89 (1,62)	n.s.
	Stress	2.05 (1,62)	n.s.
	Strain X Stress	0.24 (1,62)	n.s.

**Table 31 (cont): Results of ANOVAs on food consumption during each stress period when males and females were analyzed separately.**

<b>Females</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
<b>Stress Days 1-3</b>	Strain	0.01 (1,81)	n.s.
	Stress	1.85 (1,81)	n.s.
	Strain X Stress	0.29 (1,81)	n.s.
<b>Stress Days 3-5</b>	Strain	11.03 (1,81)	p = 0.001
	Stress	2.45 (1,81)	n.s.
	Strain X Stress	0.72 (1,81)	n.s.
<b>Stress Days 5-7</b>	Strain	0.63 (1,81)	n.s.
	Stress	5.22 (1,81)	p = 0.025
	Strain X Stress	0.18 (1,81)	n.s.
<b>Stress Days 7-9</b>	Strain	64.18 (1,81)	p < 0.001
	Stress	0.36 (1,81)	n.s.
	Strain X Stress	0.95 (1,81)	n.s.
<b>Stress Days 9-11</b>	Strain	3.94 (1,81)	p = 0.051
	Stress	0.21 (1,81)	n.s.
	Strain X Stress	0.20 (1,81)	n.s.
<b>Stress Days 11-13</b>	Strain	66.00 (1,65)	p < 0.001
	Stress	0.59 (1,65)	n.s.
	Strain X Stress	0.51 (1,65)	n.s.
<b>Stress Days 13-15</b>	Strain	53.37 (1,65)	p < 0.001
	Stress	1.06 (1,65)	n.s.
	Strain X Stress	0.00 (1,65)	n.s.
<b>Stress Days 15-17</b>	Strain	51.76 (1,65)	p < 0.001
	Stress	0.95 (1,65)	n.s.
	Strain X Stress	2.14 (1,65)	n.s.
<b>Stress Days 17-19</b>	Strain	32.38 (1,65)	p < 0.001
	Stress	3.86 (1,65)	p = 0.054
	Strain X Stress	0.26 (1,65)	n.s.
<b>Stress Days 19-21</b>	Strain	2.44 (1,65)	n.s.
	Stress	0.52 (1,65)	n.s.
	Strain X Stress	1.22 (1,65)	n.s.

**Table 32: Results of ANOVAs on food consumption during each stress period when subgroups were analyzed separately.**

<b>SD Males</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
Stress Days 1-3	Stress	0.23 (1,40)	n.s.
Stress Days 3-5	Stress	0.46 (1,40)	n.s.
Stress Days 5-7	Stress	3.12 (1,40)	p = 0.085
Stress Days 7-9	Stress	1.40 (1,40)	n.s.
Stress Days 9-11	Stress	0.03 (1,40)	n.s.
Stress Days 11-13	Stress	6.01 (1,32)	p = 0.020
Stress Days 13-15	Stress	1.02 (1,32)	n.s.
Stress Days 15-17	Stress	6.40 (1,32)	p = 0.016
Stress Days 17-19	Stress	1.69 (1,32)	n.s.
Stress Days 19-21	Stress	2.47 (1,32)	n.s.
<b>SD Females</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
Stress Days 1-3	Stress	0.02 (1,43)	n.s.
Stress Days 3-5	Stress	0.30 (1,43)	n.s.
Stress Days 5-7	Stress	2.29 (1,43)	n.s.
Stress Days 7-9	Stress	0.30 (1,43)	n.s.
Stress Days 9-11	Stress	0.03 (1,43)	n.s.
Stress Days 11-13	Stress	2.91 (1,35)	n.s.
Stress Days 13-15	Stress	0.71 (1,35)	n.s.
Stress Days 15-17	Stress	0.18 (1,35)	n.s.
Stress Days 17-19	Stress	5.05 (1,35)	p = 0.031
Stress Days 19-21	Stress	0.10 (1,35)	n.s.
<b>LE Males</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
Stress Days 1-3	Stress	0.84 (1,38)	n.s.
Stress Days 3-5	Stress	0.59 (1,38)	n.s.
Stress Days 5-7	Stress	4.17 (1,38)	p = 0.048
Stress Days 7-9	Stress	8.90 (1,38)	p = 0.005
Stress Days 9-11	Stress	2.41 (1,38)	n.s.
Stress Days 11-13	Stress	0.79 (1,30)	n.s.
Stress Days 13-15	Stress	6.39 (1,30)	p = 0.017
Stress Days 15-17	Stress	2.56 (1,30)	n.s.
Stress Days 17-19	Stress	7.69 (1,30)	p = 0.009
Stress Days 19-21	Stress	0.34 (1,30)	n.s.

<b>LE Females</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
Stress Days 1-3	Stress	0.71 (1,38)	n.s.
Stress Days 3-5	Stress	2.54 (1,38)	n.s.
Stress Days 5-7	Stress	2.83 (1,38)	n.s.
Stress Days 7-9	Stress	0.73 (1,38)	n.s.
Stress Days 9-11	Stress	0.52 (1,38)	n.s.
Stress Days 11-13	Stress	0.00 (1,30)	n.s.
Stress Days 13-15	Stress	0.39 (1,30)	n.s.
Stress Days 15-17	Stress	2.11 (1,30)	n.s.
Stress Days 17-19	Stress	0.70 (1,30)	n.s.
Stress Days 19-21	Stress	1.25 (1,30)	n.s.

**Table 33. Results of MANOVAs on Baseline locomotion variables.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 3.46 (4,156) p = 0.010	Horizontal Activity	0.77 (1,159)	n.s.
		Total Distance	0.36 (1,159)	n.s.
		Vertical Activity	0.53 (1,159)	n.s.
		Center Time	7.38 (1,159)	p = 0.007
	<b>SEX</b> 6.27 (4,156) p < 0.001	Horizontal Activity	6.39 (1,159)	p = 0.012
		Total Distance	5.48 (1,159)	p = 0.021
		Vertical Activity	0.13 (1,159)	n.s.
		Center Time	0.37 (1,159)	n.s.
	<b>STRESS</b> 0.23 (4,156) n.s.	Horizontal Activity	0.22 (1,159)	n.s.
		Total Distance	0.20 (1,159)	n.s.
		Vertical Activity	0.32 (1,159)	n.s.
		Center Time	0.39 (1,159)	n.s.
	<b>STRAIN X SEX</b> 2.86 (4,156) p = 0.025	Horizontal Activity	5.00 (1,159)	p = 0.027
		Total Distance	6.31 (1,159)	p = 0.013
		Vertical Activity	8.92 (1,159)	p = 0.003
		Center Time	3.79 (1,159)	p = 0.053
	<b>STRAIN X STRESS</b> 0.17 (4,156) n.s.	Horizontal Activity	0.00 (1,159)	n.s.
		Total Distance	0.01 (1,159)	n.s.
		Vertical Activity	0.05 (1,159)	n.s.
		Center Time	0.42 (1,159)	n.s.
	<b>SEX X STRESS</b> 0.47 (4,156) n.s.	Horizontal Activity	0.00 (1,159)	n.s.
		Total Distance	0.15 (1,159)	n.s.
		Vertical Activity	0.08 (1,159)	n.s.
		Center Time	0.54 (1,159)	n.s.
	<b>STRAIN X SEX X STRESS</b> 0.45 (4,156) n.s.	Horizontal Activity	0.60 (1,159)	n.s.
		Total Distance	1.07 (1,159)	n.s.
		Vertical Activity	0.62 (1,159)	n.s.
		Center Time	0.06 (1,159)	n.s.
SD Males	<b>STRESS</b> 0.39 (4,37) n.s.	Horizontal Activity	0.02 (1,40)	n.s.
		Total Distance	0.00 (1,40)	n.s.
		Vertical Activity	0.32 (1,40)	n.s.

		Center Time	0.33 (1,40)	n.s.
<b>SD Females</b>	<b>STRESS</b> 0.37 (4,40) n.s.	Horizontal Activity	0.35 (1,40)	n.s.
		Total Distance	0.36 (1,40)	n.s.
		Vertical Activity	0.62 (1,40)	n.s.
		Center Time	0.13 (1,40)	n.s.
<b>LE Males</b>	<b>STRESS</b> 0.37 (4,35) n.s.	Horizontal Activity	0.37 (1,38)	n.s.
		Total Distance	0.68 (1,38)	n.s.
		Vertical Activity	0.25 (1,38)	n.s.
		Center Time	0.18 (1,38)	n.s.
<b>LE Females</b>	<b>STRESS</b> 0.92 (4,35) n.s.	Horizontal Activity	0.03 (1,38)	n.s.
		Total Distance	0.18 (1,38)	n.s.
		Vertical Activity	0.02 (1,38)	n.s.
		Center Time	1.64 (1,38)	n.s.

**Table 34. Results of MANOVAs on Stress Day 1 locomotion variables.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 2.36 (4,156) p = 0.056	Horizontal Activity	3.53 (1,159)	p = 0.062
		Total Distance	2.93 (1,159)	p = 0.089
		Vertical Activity	5.22 (1,159)	p = 0.024
		Center Time	0.00 (1,159)	n.s.
	<b>SEX</b> 13.00 (4,156) p < 0.001	Horizontal Activity	8.24 (1,159)	p = 0.005
		Total Distance	6.73 (1,159)	p = 0.010
		Vertical Activity	0.09 (1,159)	n.s.
		Center Time	5.31 (1,159)	p = 0.023
	<b>STRESS</b> 3.00 (4,156) p = 0.022	Horizontal Activity	7.60 (1,159)	p = 0.007
		Total Distance	4.93 (1,159)	p = 0.045
		Vertical Activity	3.88 (1,159)	p = 0.051
		Center Time	1.93 (1,159)	n.s.
Males	<b>STRAIN X SEX</b> 0.35 (4,156) n.s.	Horizontal Activity	0.02 (1,159)	n.s.
		Total Distance	0.21 (1,159)	n.s.
		Vertical Activity	0.35 (1,159)	n.s.
		Center Time	0.11 (1,159)	n.s.
	<b>STRAIN X STRESS</b> 0.25 (4,156) n.s.	Horizontal Activity	0.10 (1,159)	n.s.
		Total Distance	0.11 (1,159)	n.s.
		Vertical Activity	0.01 (1,159)	n.s.
		Center Time	0.01 (1,159)	n.s.
	<b>SEX X STRESS</b> 0.80 (4,156) n.s.	Horizontal Activity	0.74 (1,159)	n.s.
		Total Distance	0.67 (1,159)	n.s.
		Vertical Activity	0.35 (1,159)	n.s.
		Center Time	0.39 (1,159)	n.s.
	<b>STRAIN X SEX X STRESS</b> 0.33 (4,156) n.s.	Horizontal Activity	1.31 (1,159)	n.s.
		Total Distance	1.18 (1,159)	n.s.
		Vertical Activity	1.15 (1,159)	n.s.
		Center Time	0.40 (1,159)	n.s.
	<b>STRAIN</b> 1.60 (4,75) n.s.	Horizontal Activity	2.78 (1,78)	p = 0.099
		Total Distance	3.32 (1,78)	p = 0.072
		Vertical Activity	4.29 (1,78)	p = 0.042
		Center Time	0.06 (1,78)	n.s.
	<b>STRESS</b> 3.96 (4,75) p = 0.006	Horizontal Activity	8.81 (1,78)	p = 0.004

		Total Distance	5.71 (1,78)	p = 0.019
		Vertical Activity	3.40 (1,78)	p = 0.069
		Center Time	0.29 (1,78)	n.s.
	<b>STRAIN X STRESS</b> 0.48 (4,75) n.s.	Horizontal Activity	1.04 (1,78)	n.s.
		Total Distance	1.41 (1,78)	n.s.
		Vertical Activity	0.52 (1,78)	n.s.
		Center Time	0.18 (1,78)	n.s.
Females	<b>STRAIN</b> 1.22 (4,78) n.s.	Horizontal Activity	1.20 (1,81)	n.s.
		Total Distance	0.62 (1,81)	n.s.
		Vertical Activity	1.39 (1,81)	n.s.
		Center Time	0.04 (1,81)	n.s.
	<b>STRESS</b> 0.96 (4,78) n.s.	Horizontal Activity	1.45 (1,81)	n.s.
		Total Distance	0.57 (1,81)	n.s.
		Vertical Activity	0.92 (1,81)	n.s.
		Center Time	2.03 (1,81)	n.s.
SD Males	<b>STRAIN X STRESS</b> 0.33 (4,78)	Horizontal Activity	0.44 (1,81)	n.s.
		Total Distance	0.23 (1,81)	n.s.
		Vertical Activity	0.63 (1,81)	n.s.
		Center Time	0.33 (1,81)	n.s.
	<b>STRESS</b> 1.90 (4,37) n.s.	Horizontal Activity	3.39 (1,40)	p = 0.073
		Total Distance	1.38 (1,40)	n.s.
		Vertical Activity	1.49 (1,40)	n.s.
		Center Time	0.00 (1,40)	n.s.
LE Males	<b>STRESS</b> 2.40 (4,35) n.s.	Horizontal Activity	5.36 (1,38)	p = 0.026
		Total Distance	4.20 (1,38)	p = 0.047
		Vertical Activity	2.01 (1,38)	n.s.
		Center Time	0.34 (1,38)	n.s.

**Table 35. Results of MANOVAs on Stress Day 6 locomotion variables.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 6.58 (4,156) p < 0.001	Horizontal Activity	12.84 (1,159)	p < 0.001
		Total Distance	8.99 (1,159)	p = 0.003
		Vertical Activity	9.47 (1,159)	p = 0.002
		Center Time	0.23 (1,159)	n.s.
	<b>SEX</b> 17.00 (4,156) p < 0.001	Horizontal Activity	29.00 (1,159)	p < 0.001
		Total Distance	24.06 (1,159)	p < 0.001
		Vertical Activity	1.68 (1,159)	n.s.
		Center Time	1.07 (1,159)	n.s.
	<b>STRESS</b> 1.30 (4,156) n.s.	Horizontal Activity	3.11 (1,159)	p = 0.080
		Total Distance	1.93 (1,159)	n.s.
		Vertical Activity	3.26 (1,159)	p = 0.073
		Center Time	2.00 (1,159)	n.s.
Males	<b>STRAIN X SEX</b> 0.80 (4,156) n.s.	Horizontal Activity	0.01 (1,159)	n.s.
		Total Distance	0.00 (1,159)	n.s.
		Vertical Activity	0.81 (1,159)	n.s.
		Center Time	0.46 (1,159)	n.s.
	<b>STRAIN X STRESS</b> 0.52 (4,156) n.s.	Horizontal Activity	0.09 (1,159)	n.s.
		Total Distance	0.27 (1,159)	n.s.
		Vertical Activity	0.22 (1,159)	n.s.
		Center Time	0.81 (1,159)	n.s.
	<b>SEX X STRESS</b> 0.33 (4,156) n.s.	Horizontal Activity	0.02 (1,159)	n.s.
		Total Distance	0.12 (1,159)	n.s.
		Vertical Activity	0.00 (1,159)	n.s.
		Center Time	0.22 (1,159)	n.s.
	<b>STRAIN X SEX X STRESS</b> 0.77 (4,156) n.s.	Horizontal Activity	0.27 (1,159)	n.s.
		Total Distance	0.00 (1,159)	n.s.
		Vertical Activity	0.87 (1,159)	n.s.
		Center Time	0.05 (1,159)	n.s.
	<b>STRAIN</b> 3.59 (4,75) p = 0.010	Horizontal Activity	9.87 (1,78)	p = 0.002
		Total Distance	7.61 (1,78)	p = 0.007
		Vertical Activity	10.19 (1,78)	p = 0.002
		Center Time	0.02 (1,78)	n.s.
	<b>STRESS</b> 1.07 (4,75) n.s.	Horizontal Activity	2.13 (1,78)	n.s.

		Total Distance	0.95 (1,78)	n.s.
		Vertical Activity	2.18 (1,78)	n.s.
		Center Time	0.33 (1,78)	n.s.
	<b>STRAIN X STRESS</b> 0.70 (4,75) n.s.	Horizontal Activity	0.55 (1,78)	n.s.
		Total Distance	0.29 (1,78)	n.s.
		Vertical Activity	0.02 (1,78)	n.s.
		Center Time	0.46 (1,78)	n.s.
Females	<b>STRAIN</b> 4.57 (4,78) p = 0.002	Horizontal Activity	5.00 (1,81)	p = 0.028
		Total Distance	3.34 (1,81)	p = 0.072
		Vertical Activity	1.96 (1,81)	n.s.
		Center Time	1.00 (1,81)	n.s.
	<b>STRESS</b> 0.66 (4,78) n.s.	Horizontal Activity	1.34 (1,81)	n.s.
		Total Distance	1.08 (1,81)	n.s.
		Vertical Activity	1.30 (1,81)	n.s.
		Center Time	2.58 (1,81)	n.s.
	<b>STRAIN X STRESS</b> 0.54 (4,78) n.s.	Horizontal Activity	0.02 (1,81)	n.s.
		Total Distance	0.08 (1,81)	n.s.
		Vertical Activity	0.24 (1,81)	n.s.
		Center Time	0.34 (1,81)	n.s.

**Table 36. Results of MANOVAs on Stress Day 9 locomotion variables.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 10.10 (4,156) p < 0.001	Horizontal Activity	21.93 (1,159)	p < 0.001
		Total Distance	16.22 (1,159)	p < 0.001
		Vertical Activity	26.31 (1,159)	p < 0.001
		Center Time	1.79 (1,159)	n.s.
	<b>SEX</b> 24.95 (4,156) p < 0.001	Horizontal Activity	19.59 (1,159)	p < 0.001
		Total Distance	16.67 (1,159)	p < 0.001
		Vertical Activity	1.42 (1,159)	n.s.
		Center Time	7.42 (1,159)	p = 0.007
	<b>STRESS</b> 0.79 (4,156) n.s.	Horizontal Activity	2.32 (1,159)	n.s.
		Total Distance	1.27 (1,159)	n.s.
		Vertical Activity	0.97 (1,159)	n.s.
		Center Time	1.47 (1,159)	n.s.
	<b>STRAIN X SEX</b> 0.83 (4,156) n.s.	Horizontal Activity	1.02 (1,159)	n.s.
		Total Distance	0.61 (1,159)	n.s.
		Vertical Activity	0.83 (1,159)	n.s.
		Center Time	3.18 (1,159)	p = 0.076
	<b>STRAIN X STRESS</b> 0.25 (4,156) n.s.	Horizontal Activity	0.34 (1,159)	n.s.
		Total Distance	0.261 (1,159)	n.s.
		Vertical Activity	0.72 (1,159)	n.s.
		Center Time	0.78 (1,159)	n.s.
	<b>SEX X STRESS</b> 0.32 (4,156) n.s.	Horizontal Activity	0.32 (1,159)	n.s.
		Total Distance	0.42 (1,159)	n.s.
		Vertical Activity	1.09 (1,159)	n.s.
		Center Time	0.14 (1,159)	n.s.
	<b>STRAIN X SEX X STRESS</b> 1.27 (4,156) n.s.	Horizontal Activity	3.08 (1,159)	p = 0.081
		Total Distance	2.80 (1,159)	p = 0.096
		Vertical Activity	4.25 (1,159)	p = 0.041
		Center Time	0.43 (1,159)	n.s.
Males	<b>STRAIN</b> 6.01 (4,75) p < 0.001	Horizontal Activity	20.97 (1,78)	p < 0.001
		Total Distance	16.83 (1,78)	p < 0.001
		Vertical Activity	19.50 (1,78)	p < 0.001
		Center Time	3.99 (1,78)	p = 0.049
	<b>STRESS</b> 0.69 (4,75) n.s.	Horizontal Activity	2.84 (1,78)	p = 0.096

		Total Distance	2.30 (1,78)	n.s.
		Vertical Activity	2.07 (1,78)	n.s.
		Center Time	1.04 (1,78)	n.s.
	<b>STRAIN X STRESS</b> 1.13 (4.75) n.s.	Horizontal Activity	3.55 (1,78)	p = 0.063
		Total Distance	3.49 (1,78)	p = 0.065
		Vertical Activity	4.25 (1,78)	p = 0.043
		Center Time	0.97 (1,78)	n.s.
Females	<b>STRAIN</b> 5.32 (4,78) p = 0.001	Horizontal Activity	5.56 (1,81)	p = 0.021
		Total Distance	4.08 (1,81)	p = 0.047
		Vertical Activity	9.69 (1,81)	p = 0.003
		Center Time	0.13 (1,81)	n.s.
	<b>STRESS</b> 0.40 (4,78) n.s.	Horizontal Activity	0.37 (1,81)	n.s.
		Total Distance	0.09 (1,81)	n.s.
		Vertical Activity	0.00 (1,81)	n.s.
		Center Time	0.44 (1,81)	n.s.
	<b>STRAIN X STRESS</b> 0.44 (4,78) n.s.	Horizontal Activity	0.56 (1,81)	n.s.
		Total Distance	0.52 (1,81)	n.s.
		Vertical Activity	0.74 (1,81)	n.s.
		Center Time	0.03 (1,81)	n.s.
	<b>STRESS</b> 0.18 (4,37) n.s.	Horizontal Activity	0.03 (1,40)	n.s.
		Total Distance	0.14 (1,40)	n.s.
		Vertical Activity	0.33 (1,40)	n.s.
		Center Time	0.00 (1,40)	n.s.
LE Males	<b>STRESS</b> 1.09 (4,35) n.s.	Horizontal Activity	4.40 (1,38)	p = 0.043
		Total Distance	3.60 (1,38)	p = 0.066
		Vertical Activity	4.20 (1,38)	p = 0.047
		Center Time	0.34 (1,38)	n.s.

**Table 37. Results of MANOVAs on Stress Day 19 locomotion variables.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 1.57 (4,124) n.s.	Horizontal Activity	0.09 (1,127)	n.s.
		Total Distance	0.14 (1,127)	n.s.
		Vertical Activity	0.71 (1,127)	n.s.
		Center Time	5.28 (1,127)	p = 0.023
	<b>SEX</b> 15.29 (4,124) p < 0.001	Horizontal Activity	19.09 (1,127)	p < 0.001
		Total Distance	22.38 (1,127)	p < 0.001
		Vertical Activity	0.21 (1,127)	n.s.
		Center Time	0.32 (1,127)	n.s.
	<b>STRESS</b> 1.68 (4,124) n.s.	Horizontal Activity	5.92 (1,127)	p = 0.016
		Total Distance	4.48 (1,127)	p = 0.036
		Vertical Activity	5.00 (1,127)	p = 0.027
		Center Time	1.95 (1,127)	n.s.
Males	<b>STRAIN X SEX</b> 0.38 (4,124) n.s.	Horizontal Activity	0.05 (1,127)	n.s.
		Total Distance	0.00 (1,127)	n.s.
		Vertical Activity	0.00 (1,127)	n.s.
		Center Time	0.15 (1,127)	n.s.
	<b>STRAIN X STRESS</b> 0.64 (4,124) n.s.	Horizontal Activity	1.90 (1,127)	n.s.
		Total Distance	1.68 (1,127)	n.s.
		Vertical Activity	0.56 (1,127)	n.s.
		Center Time	1.01 (1,127)	n.s.
	<b>SEX X STRESS</b> 0.50 (4,124) n.s.	Horizontal Activity	1.14 (1,127)	n.s.
		Total Distance	1.33 (1,127)	n.s.
		Vertical Activity	0.52 (1,127)	n.s.
		Center Time	1.18 (1,127)	n.s.
Males	<b>STRAIN X SEX X STRESS</b> 0.72 (4,124) n.s.	Horizontal Activity	0.94 (1,127)	n.s.
		Total Distance	1.54 (1,127)	n.s.
		Vertical Activity	1.31 (1,127)	n.s.
		Center Time	0.02 (1,127)	n.s.
	<b>STRAIN</b> 0.41 (4,59) n.s.	Horizontal Activity	0.17 (1,62)	n.s.
		Total Distance	0.06 (1,62)	n.s.
		Vertical Activity	0.33 (1,62)	n.s.
		Center Time	1.67 (1,62)	n.s.
	<b>STRESS</b> 0.43 (4,59) n.s.	Horizontal Activity	1.07 (1,62)	n.s.

		Total Distance	0.67 (1,62)	n.s.
		Vertical Activity	1.27 (1,62)	n.s.
		Center Time	0.04 (1,62)	n.s.
	<b>STRAIN X STRESS</b> 0.79 (4,59) n.s.	Horizontal Activity	0.09 (1,62)	n.s.
		Total Distance	0.00 (1,62)	n.s.
		Vertical Activity	0.08 (1,62)	n.s.
		Center Time	0.60 (1,62)	n.s.
<b>Females</b>	<b>STRAIN</b> 2.01 (4,62) n.s.	Horizontal Activity	0.00 (1,65)	n.s.
		Total Distance	0.08 (1,65)	n.s.
		Vertical Activity	0.38 (1,65)	n.s.
		Center Time	3.93 (1,65)	n.s.
	<b>STRESS</b> 1.60 (4,62) n.s.	Horizontal Activity	5.49 (1,65)	p = 0.022
		Total Distance	4.18 (1,65)	p = 0.045
		Vertical Activity	4.00 (1,65)	p = 0.050
		Center Time	3.34 (1,65)	p = 0.072
	<b>STRAIN X STRESS</b> 0.76 (4,62) n.s.	Horizontal Activity	2.46 (1,65)	n.s.
		Total Distance	2.50 (1,65)	n.s.
		Vertical Activity	1.65 (1,65)	n.s.
		Center Time	0.41 (1,65)	n.s.
	<b>STRESS</b> 2.24 (4,32) p = 0.087	Horizontal Activity	8.05 (1,35)	p = 0.008
		Total Distance	7.20 (1,35)	p = 0.011
		Vertical Activity	6.83 (1,35)	p = 0.013
		Center Time	2.19 (1,35)	n.s.
<b>LE</b> <b>Females</b>	<b>STRESS</b> 0.97 (4,27) n.s.	Horizontal Activity	0.29 (1,30)	n.s.
		Total Distance	0.09 (1,30)	n.s.
		Vertical Activity	0.20 (1,30)	n.s.
		Center Time	1.58 (1,30)	n.s.

**Table 38. Results of paired t-tests comparing locomotion variables from Stress Day 9 with locomotion variables on Stress Day 19 for each treatment group. Horizontal activity=HA, Total distance=TD, Vertical activity=VA, Center time=CT**

Strain	Treatment Group	Comparison	t value (d.f.)	p value
Sprague-Dawleys	Male - No Stress	HA Stress 9 with HA Stress 19	-2.30 (17)	p = 0.035
		TD Stress 9 with TD Stress 19	-2.60 (17)	p = 0.019
		VA Stress 9 with VA Stress 19	-2.46 (17)	p = 0.026
		CT Stress 9 with CT Stress 19	-1.82 (17)	p = 0.088
	Male - Stress	HA Stress 9 with HA Stress 19	-1.00 (17)	n.s.
		TD Stress 9 with TD Stress 19	-1.64 (17)	n.s.
		VA Stress 9 with VA Stress 19	-2.07 (17)	p = 0.055
		CT Stress 9 with CT Stress 19	-1.53 (17)	n.s.
	Female - No Stress	HA Stress 9 with HA Stress 19	-2.30 (17)	p = 0.035
		TD Stress 9 with TD Stress 19	-3.10 (17)	p = 0.007
		VA Stress 9 with VA Stress 19	-4.70 (17)	p < 0.001
		CT Stress 9 with CT Stress 19	-3.52 (17)	p = 0.003
	Female - Stress	HA Stress 9 with HA Stress 19	-0.01 (20)	n.s.
		TD Stress 9 with TD Stress 19	-0.98 (20)	n.s.
		VA Stress 9 with VA Stress 19	-1.94 (20)	p = 0.067
		CT Stress 9 with CT Stress 19	-1.35 (20)	n.s.
Long-Evans	Male - No Stress	HA Stress 9 with HA Stress 19	2.76 (16)	p = 0.015
		TD Stress 9 with TD Stress 19	1.87 (16)	p = 0.081
		VA Stress 9 with VA Stress 19	1.35 (16)	n.s.
		CT Stress 9 with CT Stress 19	0.42 (16)	n.s.
	Male - Stress	HA Stress 9 with HA Stress 19	0.74 (16)	n.s.
		TD Stress 9 with TD Stress 19	0.33 (16)	n.s.
		VA Stress 9 with VA Stress 19	0.40 (16)	n.s.
		CT Stress 9 with CT Stress 19	-1.61 (16)	n.s.
	Female - No Stress	HA Stress 9 with HA Stress 19	-0.31 (16)	n.s.
		TD Stress 9 with TD Stress 19	-1.40 (16)	n.s.
		VA Stress 9 with VA Stress 19	-0.92 (16)	n.s.
		CT Stress 9 with CT Stress 19	-1.67 (16)	n.s.
	Female - Stress	HA Stress 9 with HA Stress 19	0.74 (16)	n.s.
		TD Stress 9 with TD Stress 19	0.17 (16)	n.s.
		VA Stress 9 with VA Stress 19	0.82 (16)	n.s.
		CT Stress 9 with CT Stress 19	-0.66 (16)	n.s.

**Table 39. Results of MANOVAs on Baseline acoustic startle amplitudes.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 28.94 (2,158) p < 0.001	Startle to 120 dB	53.58 (1,159)	p < 0.001
		Startle to 110 dB	3.90 (1,159)	p = 0.050
	<b>SEX</b> 4.82 (2,158) p = 0.009	Startle to 120 dB	9.08 (1,159)	p = 0.003
		Startle to 110 dB	0.80 (1,159)	n.s.
	<b>STRESS</b> 0.12 (2,158) n.s.	Startle to 120 dB	0.09 (1,159)	n.s.
		Startle to 110 dB	0.03 (1,159)	n.s.
	<b>STRAIN X SEX</b> 3.35 (2,158) p = 0.038	Startle to 120 dB	0.30 (1,159)	n.s.
		Startle to 110 dB	6.00 (1,159)	p = 0.015
	<b>STRAIN X STRESS</b> 0.68 (2,158) n.s.	Startle to 120 dB	0.22 (1,159)	n.s.
		Startle to 110 dB	0.45 (1,159)	n.s.
SD Males	<b>SEX X STRESS</b> 1.45 (2,158) n.s.	Startle to 120 dB	0.16 (1,159)	n.s.
		Startle to 110 dB	2.63 (1,159)	n.s.
	<b>STRAIN X SEX X STRESS</b> 1.40 (2,158) n.s.	Startle to 120 dB	0.57 (1,159)	n.s.
		Startle to 110 dB	0.78 (1,159)	n.s.
SD Females	<b>STRESS</b> 0.56 (2,39) n.s.	Startle to 120 dB	0.00 (1,40)	n.s.
		Startle to 110 dB	0.88 (1,40)	n.s.
LE Males	<b>STRESS</b> 0.28 (2,37) n.s.	Startle to 120 dB	0.06 (1,43)	n.s.
		Startle to 110 dB	2.02 (1,43)	n.s.
LE Females	<b>STRESS</b> 1.85 (2,37) n.s.	Startle to 120 dB	0.05 (1,38)	n.s.
		Startle to 110 dB	0.45 (1,38)	n.s.
		Startle to 120 dB	3.58 (1,38)	p = 0.066
		Startle to 110 dB	0.00 (1,38)	n.s.

**Table 40. Results of MANOVAs on Baseline pre-pulse inhibition variables.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 3.43 (4,154) p = 0.010	120 dB w/ 68 dB pp	3.27 (1,157)	p = 0.073
		120 dB w/ 82 dB pp	0.00 (1,157)	n.s.
		110 dB w/ 68 dB pp	7.06 (1,157)	p = 0.009
		110 dB w/ 82 dB pp	0.00 (1,157)	n.s.
	<b>SEX</b> 0.58 (4,154) n.s.	120 dB w/ 68 dB pp	1.28 (1,157)	n.s.
		120 dB w/ 82 dB pp	0.34 (1,157)	n.s.
		110 dB w/ 68 dB pp	0.74 (1,157)	n.s.
		110 dB w/ 82 dB pp	1.52 (1,157)	n.s.
	<b>STRESS</b> 0.49 (4,154) n.s.	120 dB w/ 68 dB pp	1.45 (1,157)	n.s.
		120 dB w/ 82 dB pp	0.42 (1,157)	n.s.
		110 dB w/ 68 dB pp	0.95 (1,157)	n.s.
		110 dB w/ 82 dB pp	0.49 (1,157)	n.s.
	<b>STRAIN X SEX</b> 1.60 (4,154) n.s.	120 dB w/ 68 dB pp	0.07 (1,157)	n.s.
		120 dB w/ 82 dB pp	1.84 (1,157)	n.s.
		110 dB w/ 68 dB pp	0.83 (1,157)	n.s.
		110 dB w/ 82 dB pp	1.76 (1,157)	n.s.
	<b>STRAIN X STRESS</b> 2.45 (4,154) p = 0.049	120 dB w/ 68 dB pp	2.60 (1,157)	n.s.
		120 dB w/ 82 dB pp	7.68 (1,157)	p = 0.006
		110 dB w/ 68 dB pp	0.50 (1,157)	n.s.
		110 dB w/ 82 dB pp	0.00 (1,157)	n.s.
	<b>SEX X STRESS</b> 1.41 (4,154) n.s.	120 dB w/ 68 dB pp	1.154 (1,157)	n.s.
		120 dB w/ 82 dB pp	3.05 (1,157)	p = 0.083
		110 dB w/ 68 dB pp	3.84 (1,157)	p = 0.052
		110 dB w/ 82 dB pp	2.02 (1,157)	n.s.
	<b>STRAIN X SEX X STRESS</b> 1.60 (4,154) n.s.	120 dB w/ 68 dB pp	0.00 (1,157)	n.s.
		120 dB w/ 82 dB pp	0.73 (1,157)	n.s.

		110 dB w/ 68 dB pp	5.63 (1,157)	p = 0.019
		110 dB w/ 82 dB pp	0.38 (1,157)	n.s.
SD Males	<b>STRESS</b> 0.47 (4,37) n.s.	120 dB w/ 68 dB pp	1.07 (1,40)	n.s.
		120 dB w/ 82 dB pp	1.83 (1,40)	n.s.
		110 dB w/ 68 dB pp	0.14 (1,40)	n.s.
		110 dB w/ 82 dB pp	0.00 (1,40)	n.s.
SD Females	<b>STRESS</b> 2.86 (4,39) p = 0.036	120 dB w/ 68 dB pp	7.87 (1,42)	p = 0.008
		120 dB w/ 82 dB pp	5.10 (1,42)	p = 0.029
		110 dB w/ 68 dB pp	0.01 (1,42)	n.s.
		110 dB w/ 82 dB pp	4.86 (1,42)	p = 0.033
LE Males	<b>STRESS</b> 1.87 (4,35) n.s.	120 dB w/ 68 dB pp	0.37 (1,38)	n.s.
		120 dB w/ 82 dB pp	7.28 (1,38)	p = 0.010
		110 dB w/ 68 dB pp	1.01 (1,38)	n.s.
		110 dB w/ 82 dB pp	1.17 (1,38)	n.s.
LE Females	<b>STRESS</b> 2.79 (4,34) p = 0.042	120 dB w/ 68 dB pp	0.90 (1,37)	n.s.
		120 dB w/ 82 dB pp	0.04 (1,37)	n.s.
		110 dB w/ 68 dB pp	8.77 (1,37)	p = 0.005
		110 dB w/ 82 dB pp	1.96 (1,37)	n.s.

**Table 41. Results of MANOVAs on Stress Day 2 acoustic startle amplitudes with Baseline values run as covariates.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 4.77 (2,156) p = 0.010	Startle to 120 dB	0.94 (1,157)	n.s.
		Startle to 110 dB	3.66 (1,157)	p = 0.058
	<b>SEX</b> 6.55 (2,156) p = 0.002	Startle to 120 dB	7.37 (1,157)	p = 0.007
		Startle to 110 dB	0.25 (1,157)	n.s.
	<b>STRESS</b> 6.38 (2,156) p = 0.002	Startle to 120 dB	9.23 (1,157)	p = 0.003
		Startle to 110 dB	0.01 (1,157)	n.s.
	<b>STRAIN X SEX</b> 0.07 (2,156) n.s.	Startle to 120 dB	0.02 (1,157)	n.s.
		Startle to 110 dB	0.14 (1,157)	n.s.
	<b>STRAIN X STRESS</b> 1.34 (2,156) n.s.	Startle to 120 dB	2.63 (1,157)	n.s.
		Startle to 110 dB	1.14 (1,157)	n.s.
Sprague-Dawleys	<b>SEX</b> 2.92 (2,80) p = 0.058	Startle to 120 dB	3.70 (1,81)	p = 0.058
		Startle to 110 dB	0.00 (1,81)	n.s.
	<b>STRESS</b> 4.77 (2,80) p = 0.011	Startle to 120 dB	8.41 (1,81)	p = 0.005
		Startle to 110 dB	0.74 (1,81)	n.s.
	<b>SEX X STRESS</b> 0.96 (2,80) n.s.	Startle to 120 dB	0.44 (1,81)	n.s.
		Startle to 110 dB	0.33 (1,81)	n.s.
Long-Evans	<b>SEX</b> 2.53 (2,73) p = 0.087	Startle to 120 dB	1.28 (1,74)	n.s.
		Startle to 110 dB	1.44 (1,74)	n.s.
	<b>STRESS</b> 1.37 (2,73) n.s.	Startle to 120 dB	0.83 (1,74)	n.s.
		Startle to 110 dB	0.64 (1,74)	n.s.
	<b>SEX X STRESS</b> 0.15 (2,73) n.s.	Startle to 120 dB	0.13 (1,74)	n.s.

		Startle to 110 dB	0.30 (1,74)	n.s.
<b>SD Males</b>	<b>STRESS</b> 3.94 (2,37) p = 0.029	Startle to 120 dB	6.72 (1,38)	p = 0.013
		Startle to 110 dB	0.15 (1,38)	n.s.
<b>SD Females</b>	<b>STRESS</b> 2.49 (2,40) p = 0.096	Startle to 120 dB	5.03 (1,41)	p = 0.030
		Startle to 110 dB	2.68 (1,41)	n.s.

**Table 42. Results of MANOVAs on Stress Day 2 pre-pulse inhibition variables with Baseline responses as covariates.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 1.55 (4,149) n.s.	120 dB w/ 68 dB pp	1.06 (1,152)	n.s.
		120 dB w/ 82 dB pp	0.21 (1,152)	n.s.
		110 dB w/ 68 dB pp	2.29 (1,152)	n.s.
		110 dB w/ 82 dB pp	1.02 (1,152)	n.s.
	<b>SEX</b> 0.97 (4,149) n.s.	120 dB w/ 68 dB pp	0.85 (1,152)	n.s.
		120 dB w/ 82 dB pp	1.34 (1,152)	n.s.
		110 dB w/ 68 dB pp	0.18 (1,152)	n.s.
		110 dB w/ 82 dB pp	0.96 (1,152)	n.s.
	<b>STRESS</b> 3.69 (4,149) p = 0.007	120 dB w/ 68 dB pp	1.73 (1,152)	n.s.
		120 dB w/ 82 dB pp	10.15 (1,152)	p = 0.002
		110 dB w/ 68 dB pp	4.87 (1,152)	p = 0.029
		110 dB w/ 82 dB pp	6.00 (1,152)	p = 0.016
	<b>STRAIN X SEX</b> 2.59 (4,149) p = 0.039	120 dB w/ 68 dB pp	0.04 (1,152)	n.s.
		120 dB w/ 82 dB pp	0.30 (1,152)	n.s.
		110 dB w/ 68 dB pp	5.03 (1,152)	p = 0.026
		110 dB w/ 82 dB pp	2.81 (1,152)	p = 0.096
	<b>STRAIN X STRESS</b> 0.47 (4,149) n.s.	120 dB w/ 68 dB pp	0.35 (1,152)	n.s.
		120 dB w/ 82 dB pp	1.21 (1,152)	n.s.
		110 dB w/ 68 dB pp	0.58 (1,152)	n.s.
		110 dB w/ 82 dB pp	0.03 (1,152)	n.s.
	<b>SEX X STRESS</b> 1.08 (4,149) n.s.	120 dB w/ 68 dB pp	0.03 (1,152)	n.s.
		120 dB w/ 82 dB pp	0.34 (1,152)	n.s.
		110 dB w/ 68 dB pp	3.92 (1,152)	p = 0.049
		110 dB w/ 82 dB pp	0.16 (1,152)	n.s.
	<b>STRAIN X SEX X STRESS</b> 1.76 (4,149) n.s.	120 dB w/ 68 dB pp	0.24 (1,152)	n.s.

		120 dB w/ 82 dB pp	0.87 (1,152)	n.s.
		110 dB w/ 68 dB pp	2.93 (1,152)	p = 0.089
		110 dB w/ 82 dB pp	0.68 (1,152)	n.s.
Sprague-Dawleys	<b>SEX</b> 1.71 (4,75) n.s.	120 dB w/ 68 dB pp	0.52 (1,78)	n.s.
		120 dB w/ 82 dB pp	1.40 (1,78)	n.s.
		110 dB w/ 68 dB pp	1.53 (1,78)	n.s.
		110 dB w/ 82 dB pp	0.73 (1,78)	n.s.
	<b>STRESS</b> 3.44 (4,75) p = 0.012	120 dB w/ 68 dB pp	1.96 (1,78)	n.s.
		120 dB w/ 82 dB pp	8.74 (1,78)	p = 0.004
		110 dB w/ 68 dB pp	5.31 (1,78)	p = 0.024
		110 dB w/ 82 dB pp	6.07 (1,78)	p = 0.016
Long-Evans	<b>SEX X STRESS</b> 0.53 (4,75) n.s.	120 dB w/ 68 dB pp	0.21 (1,78)	n.s.
		120 dB w/ 82 dB pp	0.02 (1,78)	n.s.
		110 dB w/ 68 dB pp	0.04 (1,78)	n.s.
		110 dB w/ 82 dB pp	1.80 (1,78)	n.s.
	<b>SEX</b> 1.58 (4,67) n.s.	120 dB w/ 68 dB pp	0.74 (1,70)	n.s.
		120 dB w/ 82 dB pp	0.22 (1,70)	n.s.
		110 dB w/ 68 dB pp	3.86 (1,70)	p = 0.053
		110 dB w/ 82 dB pp	1.80 (1,70)	n.s.
	<b>STRESS</b> 0.95 (4,67) n.s.	120 dB w/ 68 dB pp	0.47 (1,70)	n.s.
		120 dB w/ 82 dB pp	2.15 (1,70)	n.s.
		110 dB w/ 68 dB pp	0.89 (1,70)	n.s.
		110 dB w/ 82 dB pp	2.77 (1,70)	n.s.
	<b>SEX X STRESS</b> 1.71 (4,67) n.s.	120 dB w/ 68 dB pp	0.03 (1,70)	n.s.
		120 dB w/ 82 dB pp	0.55 (1,70)	n.s.
		110 dB w/ 68 dB pp	4.76 (1,70)	p = 0.033
		110 dB w/ 82 dB pp	0.02 (1,70)	n.s.
SD Males	<b>STRESS</b> 1.25 (4,33) n.s.	120 dB w/ 68 dB pp	1.07 (1,36)	n.s.
		120 dB w/ 82 dB pp	1.83 (1,36)	p = 0.066

		110 dB w/ 68 dB pp	0.14 (1,36)	n.s.
		110 dB w/ 82 dB pp	0.00 (1.36)	n.s.
<b>SD Females</b>	<b>STRESS</b> 3.0 (4,35) p = 0.033	120 dB w/ 68 dB pp	7.87 (1,38)	n.s.
		120 dB w/ 82 dB pp	5.10 (1,38)	p = 0.009
		110 dB w/ 68 dB pp	0.01 (1,38)	n.s.
		110 dB w/ 82 dB pp	4.86 (1,38)	p = 0.002
<b>LE Males</b>	<b>STRESS</b> 1.38 (4,30) n.s.	120 dB w/ 68 dB pp	0.37 (1,33)	n.s.
		120 dB w/ 82 dB pp	7.28 (1,33)	n.s.
		110 dB w/ 68 dB pp	1.01 (1,33)	n.s.
		110 dB w/ 82 dB pp	1.17 (1,33)	n.s.
<b>LE Females</b>	<b>STRESS</b> 2.53 (4,30) p = 0.061	120 dB w/ 68 dB pp	0.90 (1,33)	n.s.
		120 dB w/ 82 dB pp	0.04 (1,33)	n.s.
		110 dB w/ 68 dB pp	8.77 (1,33)	p = 0.048
		110 dB w/ 82 dB pp	1.96 (1,33)	p = 0.017

**Table 43. Results of MANOVAs on Stress Day 7 acoustic startle amplitudes with Baseline values run as covariates.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 3.12 (2,156) p = 0.047	Startle to 120 dB	3.72 (1,157)	p = 0.055
		Startle to 110 dB	5.83 (1,157)	p = 0.017
	<b>SEX</b> 0.70 (2,156) n.s.	Startle to 120 dB	1.40 (1,157)	n.s.
		Startle to 110 dB	0.55 (1,157)	n.s.
	<b>STRESS</b> 1.65 (2,156) n.s.	Startle to 120 dB	2.96 (1,157)	p = 0.087
		Startle to 110 dB	0.24 (1,157)	n.s.
	<b>STRAIN X SEX</b> 0.19 (2,156) n.s.	Startle to 120 dB	0.38 (1,157)	n.s.
		Startle to 110 dB	0.11 (1,157)	n.s.
	<b>STRAIN X STRESS</b> 2.13 (2,156) n.s.	Startle to 120 dB	2.04 (1,157)	n.s.
		Startle to 110 dB	4.19 (1,157)	p = 0.042
Sprague-Dawleys	<b>SEX</b> 0.18 (2,80) n.s.	Startle to 120 dB	0.36 (1,81)	n.s.
		Startle to 110 dB	0.11 (1,81)	n.s.
	<b>STRESS</b> 2.37 (2,80) p = 0.100	Startle to 120 dB	4.69 (1,81)	p = 0.033
		Startle to 110 dB	2.58 (1,81)	n.s.
	<b>SEX X STRESS</b> 0.73 (2,80) n.s.	Startle to 120 dB	1.40 (1,81)	n.s.
		Startle to 110 dB	0.91 (1,81)	n.s.
Long-Evans	<b>SEX</b> 0.91 (2,73) n.s.	Startle to 120 dB	1.70 (1,74)	n.s.
		Startle to 110 dB	0.11 (1,74)	n.s.
	<b>STRESS</b> 1.42 (2,73) n.s.	Startle to 120 dB	0.00 (1,74)	n.s.
		Startle to 110 dB	2.17 (1,74)	n.s.
	<b>SEX X STRESS</b> 0.86 (2,73) n.s.	Startle to 120 dB	0.91 (1,74)	n.s.
		Startle to 110 dB	0.09 (1,74)	n.s.
SD Males	<b>STRESS</b> 1.84 (2,37) n.s.	Startle to 120 dB	3.75 (1,38)	p = 0.060
		Startle to 110 dB	2.35 (1,38)	n.s.
SD Females	<b>STRESS</b> 0.57 (2,40) n.s.	Startle to 120 dB	0.52 (1,41)	n.s.
		Startle to 110 dB	1.05 (1,41)	n.s.

**Table 44. Results of MANOVAs on Stress Day 7 pre-pulse inhibition variables with Baseline responses as covariates.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	STRAIN 4.70 (4,149) p = 0.001	120 dB w/ 68 dB pp	7.08 (1,152)	p = 0.009
		120 dB w/ 82 dB pp	0.38 (1,152)	n.s.
		110 dB w/ 68 dB pp	15.56 (1,152)	p < 0.001
		110 dB w/ 82 dB pp	0.06 (1,152)	n.s.
	SEX 4.47 (4,149) p = 0.002	120 dB w/ 68 dB pp	7.79 (1,152)	p = 0.006
		120 dB w/ 82 dB pp	4.16 (1,152)	p = 0.043
		110 dB w/ 68 dB pp	11.68 (1,152)	p = 0.001
		110 dB w/ 82 dB pp	0.32 (1,152)	n.s.
	STRESS 1.26 (4,149) n.s.	120 dB w/ 68 dB pp	1.38 (1,152)	n.s.
		120 dB w/ 82 dB pp	2.34 (1,152)	n.s.
		110 dB w/ 68 dB pp	2.63 (1,152)	n.s.
		110 dB w/ 82 dB pp	2.59 (1,152)	n.s.
	STRAIN X SEX 1.17 (4,149) n.s.	120 dB w/ 68 dB pp	0.87 (1,152)	n.s.
		120 dB w/ 82 dB pp	2.76 (1,152)	p = 0.099.
		110 dB w/ 68 dB pp	0.24 (1,152)	n.s.
		110 dB w/ 82 dB pp	0.23 (1,152)	n.s.
	STRAIN X STRESS 1.11 (4,149) n.s.	120 dB w/ 68 dB pp	1.99 (1,152)	n.s.
		120 dB w/ 82 dB pp	2.44 (1,152)	n.s.
		110 dB w/ 68 dB pp	0.69 (1,152)	n.s.
		110 dB w/ 82 dB pp	0.06 (1,152)	n.s.
	SEX X STRESS 0.56 (4,149) n.s.	120 dB w/ 68 dB pp	0.41 (1,152)	n.s.
		120 dB w/ 82 dB pp	0.89 (1,152)	n.s.
		110 dB w/ 68 dB pp	0.00 (1,152)	n.s.
		110 dB w/ 82 dB pp	1.63 (1,152)	n.s.
	STRAIN X SEX X STRESS 0.75 (4,149) n.s.	120 dB w/ 68 dB pp	0.00 (1,152)	n.s.
		120 dB w/ 82 dB pp	1.02 (1,152)	n.s.
		110 dB w/ 68 dB pp	1.66 (1,152)	n.s.
		110 dB w/ 82 dB pp	1.52 (1,152)	n.s.
Sprague-Dawleys	SEX 3.72 (4,74) p = 0.008	120 dB w/ 68 dB pp	1.58 (1,77)	n.s.
		120 dB w/ 82 dB pp	7.25 (1,77)	p = 0.009

		110 dB w/ 68 dB pp	6.64 (1,77)	p = 0.012
		110 dB w/ 82 dB pp	0.18 (1,77)	n.s.
<b>STRESS</b> 0.44 (4.74) n.s.		120 dB w/ 68 dB pp	0.05 (1,77)	n.s.
		120 dB w/ 82 dB pp	0.00 (1,77)	n.s.
		110 dB w/ 68 dB pp	0.03 (1,77)	n.s.
		110 dB w/ 82 dB pp	1.03 (1,77)	n.s.
<b>SEX X STRESS</b> 0.36 (4.74) n.s.		120 dB w/ 68 dB pp	0.21 (1,77)	n.s.
		120 dB w/ 82 dB pp	0.00 (1,77)	n.s.
		110 dB w/ 68 dB pp	0.76 (1,77)	n.s.
		110 dB w/ 82 dB pp	0.01 (1,77)	n.s.
<b>Long-Evans</b>	<b>SEX</b> 2.74 (4.68) p = 0.036	120 dB w/ 68 dB pp	7.66 (1,71)	p = 0.007
		120 dB w/ 82 dB pp	0.03 (1,71)	n.s.
		110 dB w/ 68 dB pp	6.91 (1,71)	p = 0.010
		110 dB w/ 82 dB pp	0.00 (1,71)	n.s.
<b>STRESS</b> 1.84 (4.68) n.s.		120 dB w/ 68 dB pp	4.18 (1,71)	p = 0.045
		120 dB w/ 82 dB pp	3.59 (1,71)	p = 0.062
		110 dB w/ 68 dB pp	2.82 (1,71)	p = 0.097
		110 dB w/ 82 dB pp	1.42 (1,71)	n.s.
<b>SEX X STRESS</b> 0.56 (4.68) n.s.		120 dB w/ 68 dB pp	0.54 (1,71)	n.s.
		120 dB w/ 82 dB pp	1.19 (1,71)	n.s.
		110 dB w/ 68 dB pp	0.94 (1,71)	n.s.
		110 dB w/ 82 dB pp	1.46 (1,71)	n.s.
<b>LE Males</b>	<b>STRESS</b> 0.35 (4.31) n.s.	120 dB w/ 68 dB pp	0.81 (1,34)	n.s.
		120 dB w/ 82 dB pp	1.13 (1,34)	n.s.
		110 dB w/ 68 dB	0.42 (1,34)	n.s.
		110 dB w/ 82 dB pp	0.24 (1,34)	n.s.
<b>LE Females</b>	<b>STRESS</b> 2.41 (4.30) n.s.	120 dB w/ 68 dB pp	4.98 (1,33)	p = 0.033
		120 dB w/ 82 dB pp	1.24 (1,33)	n.s.
		110 dB w/ 68 dB pp	0.99 (1,33)	n.s.
		110 dB w/ 82 dB pp	0.00 (1,33)	n.s.

**Table 45. Results of MANOVAs on Stress Day 10 acoustic startle amplitudes with Baseline values run as covariates.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	STRAIN 4.18 (2,156) p = 0.017	Startle to 120 dB	4.34 (1,157)	p = 0.039
		Startle to 110 dB	7.60 (1,157)	p = 0.007
	SEX 4.61 (2,156) p = 0.011	Startle to 120 dB	9.26 (1,157)	p = 0.003
		Startle to 110 dB	2.27 (1,157)	n.s.
	STRESS 1.15 (2,156) n.s.	Startle to 120 dB	2.05 (1,157)	n.s.
		Startle to 110 dB	1.25 (1,157)	n.s.
	STRAIN X SEX 0.00 (2,156) n.s.	Startle to 120 dB	0.00 (1,157)	n.s.
		Startle to 110 dB	0.01 (1,157)	n.s.
	STRAIN X STRESS 4.67 (2,156) p = 0.011	Startle to 120 dB	9.31 (1,157)	p = 0.003
		Startle to 110 dB	2.84 (1,157)	p = 0.094
Sprague-Dawleys	SEX 1.71 (2,80) n.s.	Startle to 120 dB	0.11 (1,157)	n.s.
		Startle to 110 dB	1.93 (1,157)	n.s.
	STRESS 3.93 (2,80) p = 0.024	Startle to 120 dB	7.60 (1,81)	p = 0.007
		Startle to 110 dB	3.25 (1,81)	p = 0.075
	SEX X STRESS 0.40 (2,80) n.s.	Startle to 120 dB	0.17 (1,81)	n.s.
		Startle to 110 dB	0.26 (1,81)	n.s.
	SEX 2.65 (2,73) p = 0.078	Startle to 120 dB	5.16 (1,74)	p = 0.026
		Startle to 110 dB	0.41 (1,74)	n.s.
Long-Evans	STRESS 1.07 (2,73) n.s.	Startle to 120 dB	2.14 (1,74)	n.s.
		Startle to 110 dB	0.27 (1,74)	n.s.
	SEX X STRESS 1.51 (2,73) n.s.	Startle to 120 dB	0.00 (1,74)	n.s.
		Startle to 110 dB	2.47 (1,74)	n.s.
	SD Males STRESS 2.44 (2,37) n.s.	Startle to 120 dB	4.84 (1,38)	p = 0.034
		Startle to 110 dB	1.18 (1,38)	n.s.
SD Females	STRESS 3.27 (2,40) p = 0.049	Startle to 120 dB	5.30 (1,41)	p = 0.026
		Startle to 110 dB	5.40 (1,41)	p = 0.025

**Table 46. Results of MANOVAs on Stress Day 10 pre-pulse inhibition variables with Baseline responses as covariates.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 1.83 (4,149) n.s.	120 dB w/ 68 dB pp	4.34 (1,152)	p = 0.039
		120 dB w/ 82 dB pp	1.04 (1,152)	n.s.
		110 dB w/ 68 dB pp	4.806 (1,152)	p = 0.030
		110 dB w/ 82 dB pp	2.00 (1,152)	n.s.
	<b>SEX</b> 2.32 (4,149) p = 0.060	120 dB w/ 68 dB pp	5.51 (1,152)	p = 0.020
		120 dB w/ 82 dB pp	3.91 (1,152)	p = 0.050
		110 dB w/ 68 dB pp	1.50 (1,152)	n.s.
		110 dB w/ 82 dB pp	2.61 (1,152)	n.s.
	<b>STRESS</b> 2.99 (4,149) p = 0.021	120 dB w/ 68 dB pp	2.94 (1,152)	p = 0.088
		120 dB w/ 82 dB pp	2.36 (1,152)	n.s.
		110 dB w/ 68 dB pp	8.28 (1,152)	p = 0.005
		110 dB w/ 82 dB pp	5.31 (1,152)	p = 0.023
	<b>STRAIN X SEX</b> 2.00 (4,149) p = 0.098	120 dB w/ 68 dB pp	1.38 (1,152)	n.s.
		120 dB w/ 82 dB pp	2.25 (1,152)	n.s.
		110 dB w/ 68 dB pp	1.76 (1,152)	n.s.
		110 dB w/ 82 dB pp	2.00 (1,152)	n.s.
	<b>STRAIN X STRESS</b> 2.21 (4,149) p = 0.070	120 dB w/ 68 dB pp	0.28 (1,152)	n.s.
		120 dB w/ 82 dB pp	2.80 (1,152)	p = 0.096
		110 dB w/ 68 dB pp	4.02 (1,152)	p = 0.047
		110 dB w/ 82 dB pp	0.39 (1,152)	n.s.
	<b>SEX X STRESS</b> 1.45 (4,149) n.s.	120 dB w/ 68 dB pp	0.01 (1,152)	n.s.
		120 dB w/ 82 dB pp	1.29 (1,152)	n.s.
		110 dB w/ 68 dB pp	2.41 (1,152)	n.s.
		110 dB w/ 82 dB pp	1.51 (1,152)	n.s.
	<b>STRAIN X SEX X STRESS</b> 1.67 (4,149) n.s.	120 dB w/ 68 dB pp	1.16 (1,152)	n.s.

		120 dB w/ 82 dB pp	0.31 (1,152)	n.s.
		110 dB w/ 68 dB pp	0.03 (1,152)	n.s.
		110 dB w/ 82 dB pp	2.81 (1,152)	p = 0.096
<b>Sprague-Dawleys</b>	<b>SEX</b> 1.81 (4,74) n.s.	120 dB w/ 68 dB pp	0.52 (1,77)	n.s.
		120 dB w/ 82 dB pp	5.39 (1,77)	p = 0.023
		110 dB w/ 68 dB pp	2.75 (1,77)	n.s.
		110 dB w/ 82 dB pp	3.90 (1,77)	p = 0.052
	<b>STRESS</b> 1.34 (4,74) n.s.	120 dB w/ 68 dB pp	0.89 (1,77)	n.s.
		120 dB w/ 82 dB pp	4.43 (1,77)	p = 0.040
		110 dB w/ 68 dB pp	0.78 (1,77)	n.s.
		110 dB w/ 82 dB pp	3.38 (1,77)	p = 0.070
	<b>SEX X STRESS</b> 1.13 (4,74) n.s.	120 dB w/ 68 dB pp	0.68 (1,77)	n.s.
		120 dB w/ 82 dB pp	0.13 (1,77)	n.s.
		110 dB w/ 68 dB pp	0.97 (1,77)	n.s.
		110 dB w/ 82 dB pp	2.864 (1,77)	p = 0.095
<b>Long-Evans</b>	<b>SEX</b> 3.00 (4,68) p = 0.025	120 dB w/ 68 dB pp	6.58 (1,71)	p = 0.012
		120 dB w/ 82 dB pp	0.15 (1,71)	n.s.
		110 dB w/ 68 dB pp	0.06 (1,71)	n.s.
		110 dB w/ 82 dB pp	0.08 (1,71)	n.s.
	<b>STRESS</b> 3.56 (4,68) p = 0.011	120 dB w/ 68 dB pp	3.18 (1,71)	p = 0.079
		120 dB w/ 82 dB pp	0.16 (1,71)	n.s.
		110 dB w/ 68 dB pp	12.49 (1,71)	p = 0.001
		110 dB w/ 82 dB pp	1.32 (1,71)	n.s.
	<b>SEX X STRESS</b> 1.70 (4,68) n.s.	120 dB w/ 68 dB pp	0.59 (1,71)	n.s.
		120 dB w/ 82 dB pp	2.16 (1,71)	n.s.
		110 dB w/ 68 dB pp	0.80 (1,71)	n.s.
		110 dB w/ 82 dB pp	0.03 (1,71)	n.s.
<b>SD Males</b>	<b>STRESS</b> 0.95 (4,32) n.s.	120 dB w/ 68 dB pp	0.00 (1,35)	n.s.
		120 dB w/ 82 dB pp	1.34 (1,35)	n.s.

		110 dB w/ 68 dB pp	0.04 (1,35)	n.s.
		110 dB w/ 82 dB pp	3.68 (1,35)	p = 0.063
<b>SD Females</b>	<b>STRESS</b> 1.59 (4,35) n.s.	120 dB w/ 68 dB pp	0.94 (1,38)	n.s.
		120 dB w/ 82 dB pp	4.94 (1,38)	p = 0.032
		110 dB w/ 68 dB pp	2.06 (1,38)	n.s.
		110 dB w/ 82 dB pp	0.39 (1,38)	n.s.
<b>LE Males</b>	<b>STRESS</b> 0.97 (4,31) n.s.	120 dB w/ 68 dB pp	1.34 (1,34)	n.s.
		120 dB w/ 82 dB pp	2.79 (1,34)	n.s.
		110 dB w/ 68 dB pp	2.38 (1,34)	n.s.
		110 dB w/ 82 dB pp	2.13 (1,34))	n.s.
<b>LE Females</b>	<b>STRESS</b> 2.48 (4,30) p = 0.065	120 dB w/ 68 dB pp	0.52 (1,33)	n.s.
		120 dB w/ 82 dB pp	0.89 (1,33)	n.s.
		110 dB w/ 68 dB pp	6.71 (1,33)	p = 0.014
		110 dB w/ 82 dB pp	1.10 (1,33)	n.s.

**Table 47. Results of MANOVAs on Stress Day 20 acoustic startle amplitudes with Baseline values run as covariates.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 10.91 (2,124) p < 0.001	Startle to 120 dB	0.19 (1,125)	n.s.
		Startle to 110 dB	16.0 (1,125)	p < 0.001
	<b>SEX</b> 22.54 (2,124) p < 0.001	Startle to 120 dB	42.93 (1,125)	p < 0.001
		Startle to 110 dB	7.06 (1,125)	p = 0.009
	<b>STRESS</b> 3.29 (2,124) p = 0.041	Startle to 120 dB	4.66 (1,125)	p = 0.033
		Startle to 110 dB	0.03 (1,125)	n.s.
	<b>STRAIN X SEX</b> 0.89 (2,124) n.s.	Startle to 120 dB	1.16 (1,125)	n.s.
		Startle to 110 dB	0.00 (1,125)	n.s.
	<b>STRAIN X STRESS</b> 4.14 (2,124) p = 0.018	Startle to 120 dB	3.88 (1,125)	p = 0.051
		Startle to 110 dB	8.26 (1,125)	p = 0.005
Sprague-Dawleys	<b>SEX</b> 12.08 (2,64) p < 0.001	Startle to 120 dB	3.46 (1,125)	p = 0.065
		Startle to 110 dB	0.65 (1,125)	n.s.
	<b>STRESS</b> 3.48 (2,64) p = 0.037	Startle to 120 dB	2.56 (1,125)	n.s.
		Startle to 110 dB	1.70 (1,125)	n.s.
	<b>SEX X STRESS</b> 2.19 (2,64) n.s.	Startle to 120 dB	4.44 (1,65)	p = 0.039
		Startle to 110 dB	2.03 (1,65)	n.s.
Long-Evans	<b>SEX</b> 9.09 (2,57) p < 0.001	Startle to 120 dB	17.92 (1,58)	p < 0.001
		Startle to 110 dB	2.74 (1,58)	n.s.
	<b>STRESS</b> 3.07 (2,57) p = 0.054	Startle to 120 dB	0.03 (1,58)	n.s.
		Startle to 110 dB	4.78 (1,58)	p = 0.033
	<b>SEX X STRESS</b> 0.41 (2,57) n.s.	Startle to 120 dB	0.38 (1,58)	n.s.

		Startle to 110 dB	0.06 (1,58)	n.s.
<b>SD Males</b>	<b>STRESS</b> 3.56 (2,29) p = 0.041	Startle to 120 dB	6.77 (1,30)	p = 0.014
		Startle to 110 dB	5.05 (1,30)	p = 0.032
<b>SD Females</b>	<b>STRESS</b> 0.87 (2,32) n.s.	Startle to 120 dB	0.23 (1,33)	n.s.
		Startle to 110 dB	1.48 (1,33)	n.s.
<b>LE Males</b>	<b>STRESS</b> 1.98 (2,27) n.s.	Startle to 120 dB	0.05 (1,28)	n.s.
		Startle to 110 dB	2.07 (1,28)	n.s.
<b>LE Females</b>	<b>STRESS</b> 1.46 (2,27) n.s.	Startle to 120 dB	0.00 (1,28)	n.s.
		Startle to 110 dB	2.80 (1,28)	n.s.

**Table 48. Results of MANOVAs on Stress Day 20 pre-pulse inhibition variables with Baseline responses as covariates.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 1.85 (4,118) n.s.	120 dB w/ 68 dB pp	2.67 (1,121)	n.s.
		120 dB w/ 82 dB pp	0.05 (1,121)	n.s.
		110 dB w/ 68 dB pp	1.52 (1,121)	n.s.
		110 dB w/ 82 dB pp	3.56 (1,121)	p = 0.062
	<b>SEX</b> 0.13 (4,118) n.s.	120 dB w/ 68 dB pp	0.01 (1,121)	n.s.
		120 dB w/ 82 dB pp	0.07 (1,121)	n.s.
		110 dB w/ 68 dB pp	0.38 (1,121)	n.s.
		110 dB w/ 82 dB pp	0.00 (1,121)	n.s.
	<b>STRESS</b> 2.04 (4,118) p = 0.094	120 dB w/ 68 dB pp	0.64 (1,121)	n.s.
		120 dB w/ 82 dB pp	0.07 (1,121)	n.s.
		110 dB w/ 68 dB pp	7.54 (1,121)	p = 0.007
		110 dB w/ 82 dB pp	0.19 (1,121)	n.s.
	<b>STRAIN X SEX</b> 2.14 (4,118) p = 0.081	120 dB w/ 68 dB pp	0.14 (1,121)	n.s.
		120 dB w/ 82 dB pp	1.56 (1,121)	n.s.
		110 dB w/ 68 dB pp	0.33 (1,121)	n.s.
		110 dB w/ 82 dB pp	2.82 (1,121)	p = 0.096
	<b>STRAIN X STRESS</b> 1.67 (4,118) n.s.	120 dB w/ 68 dB pp	0.64 (1,121)	n.s.
		120 dB w/ 82 dB pp	2.11 (1,121)	n.s.
		110 dB w/ 68 dB pp	0.14 (1,121)	n.s.
		110 dB w/ 82 dB pp	6.16 (1,121)	p = 0.014
	<b>SEX X STRESS</b> 0.62 (4,118) n.s.	120 dB w/ 68 dB pp	0.51 (1,121)	n.s.
		120 dB w/ 82 dB pp	0.49 (1,121)	n.s.
		110 dB w/ 68 dB pp	0.87 (1,121)	n.s.
		110 dB w/ 82 dB pp	0.21 (1,121)	n.s.
	<b>STRAIN X SEX X STRESS</b> 1.21 (4,118) n.s.	120 dB w/ 68 dB pp	0.27 (1,121)	n.s.

		120 dB w/ 82 dB pp	3.49 (1,121)	p = 0.064
		110 dB w/ 68 dB pp	0.13 (1,121)	n.s.
		110 dB w/ 82 dB pp	0.00 (1,121)	n.s.
Sprague-Dawleys	<b>SEX</b> 0.61 (4,59) n.s.	120 dB w/ 68 dB pp	0.25 (1,62)	n.s.
		120 dB w/ 82 dB pp	0.43 (1,62)	n.s.
		110 dB w/ 68 dB pp	0.02 (1,62)	n.s.
		110 dB w/ 82 dB pp	1.04 (1,62)	n.s.
	<b>STRESS</b> 1.97 (4,59) n.s.	120 dB w/ 68 dB pp	0.94 (1,62)	n.s.
		120 dB w/ 82 dB pp	1.21 (1,62)	n.s.
		110 dB w/ 68 dB pp	4.26 (1,62)	p = 0.043
		110 dB w/ 82 dB pp	2.14 (1,62)	n.s.
Long-Evans	<b>SEX</b> 2.51 (4,52) p = 0.053	120 dB w/ 68 dB pp	0.00 (1,62)	n.s.
		120 dB w/ 82 dB pp	3.21 (1,62)	p = 0.078
		110 dB w/ 68 dB pp	0.78 (1,62)	n.s.
		110 dB w/ 82 dB pp	0.09 (1,62)	n.s.
	<b>STRESS</b> 2.49 (4,52) p = 0.055	120 dB w/ 68 dB pp	0.05 (1,55)	n.s.
		120 dB w/ 82 dB pp	1.06 (1,55)	n.s.
		110 dB w/ 68 dB pp	1.17 (1,55)	n.s.
		110 dB w/ 82 dB pp	1.56 (1,55)	n.s.
	<b>SEX X STRESS</b> 0.21 (4,52) n.s.	120 dB w/ 68 dB pp	0.73 (1,55)	n.s.
		120 dB w/ 82 dB pp	0.51 (1,55)	n.s.
		110 dB w/ 68 dB pp	3.45 (1,55)	p = 0.069
		110 dB w/ 82 dB pp	5.56 (1,55)	p = 0.022
SD Males	<b>STRESS</b> 0.97 (4,25) n.s.	120 dB w/ 68 dB pp	0.45 (1,55)	n.s.
		120 dB w/ 82 dB pp	0.59 (1,55)	n.s.
		110 dB w/ 68 dB pp	0.01 (1,55)	n.s.
		110 dB w/ 82 dB pp	0.16 (1,55)	n.s.
		120 dB w/ 68 dB pp	0.09 (1,28)	n.s.
		120 dB w/ 82 dB pp	0.62 (1,28)	n.s.

		110 dB w/ 68 dB pp	0.26 (1,28)	n.s.
		110 dB w/ 82 dB pp	1.46 (1,28)	n.s.
<b>SD Females</b>	<b>STRESS</b> 2.60 (4,27) p = 0.059	120 dB w/ 68 dB pp	0.00 (1,30)	n.s.
		120 dB w/ 82 dB pp	2.97 (1,30)	p = 0.095
		110 dB w/ 68 dB pp	7.93 (1,30)	p = 0.008
		110 dB w/ 82 dB pp	0.22 (1,30)	n.s.
<b>LE Males</b>	<b>STRESS</b> 2.42 (4,23) p = 0.078	120 dB w/ 68 dB pp	0.43 (1,26)	n.s.
		120 dB w/ 82 dB pp	0.09 (1,26)	n.s.
		110 dB w/ 68 dB pp	5.16 (1,26)	p = 0.032
		110 dB w/ 82 dB pp	1.97 (1,26))	n.s.
<b>LE Females</b>	<b>STRESS</b> 0.41 (4,22) n.s.	120 dB w/ 68 dB pp	0.86 (1,25)	n.s.
		120 dB w/ 82 dB pp	0.16 (1,25)	n.s.
		110 dB w/ 68 dB pp	0.01 (1,25)	n.s.
		110 dB w/ 82 dB pp	1.60 (1,25)	n.s.

**Table 49:** Results from Wilcoxon Signed Ranks Test on passive avoidance training latencies compared to testing latencies

Group Tested	Effect	Z value (d.f.)	p value
All animals	Time	-10.747 (156)	p < 0.001
SD Males-No Stress	Time	-3.621 (17)	p < 0.001
SD Males-Stress	Time	-3.743 (19)	p < 0.001
SD Females-No Stress	Time	-3.920 (20)	p < 0.001
SD Females-Stress	Time	-4.200 (24)	p < 0.001
LE Males-No Stress	Time	-3.680 (18)	p < 0.001
LE Males-Stress	Time	-3.920 (20)	p < 0.001
LE Females-No Stress	Time	-3.783 (19)	p < 0.001
LE Females-Stress	Time	-3.783 (19)	p < 0.001

**Table 50:** ANOVAs on transformed (raised to the power of 0.295) passive avoidance training latencies

Group Tested	Effect	F value (d.f.)	p value
All animals	Strain	0.173 (1,148)	n.s.
	Sex	5.108 (1,148)	p=0.025
	Stress	3.296 (1,148)	p=0.071
	Strain X Sex	0.682 (1,148)	n.s.
	Strain X Stress	0.046 (1,148)	n.s.
	Sex X Stress	2.036 (1,148)	n.s.
	Strain X Sex X Stress	5.908 (1,148)	p=0.016
Sprague-Dawleys	Sex	1.207 (1,76)	n.s.
	Stress	1.504 (1,76)	n.s.
	Sex X Stress	0.591 (1,76)	n.s.
Long-Evans	Sex	4.103 (1,72)	p=0.047
	Stress	1.777 (1,72)	n.s.
	Sex X Stress	6.412 (1,72)	p=0.014
LE Males	Stress	1.052 (1,36)	n.s.
LE Females	Stress	5.678 (1,36)	p=0.023

**Table 51: Kruskal-Wallis nonparametric ANOVAs on passive avoidance testing latencies.**

Group Tested	Effect	Chi Square (d.f.)	p value
All animals	Strain	5.898 (1,156)	p=0.015
	Sex	3.380 (1,156)	p=0.066
	Stress	0.171 (1,156)	n.s.
Sprague-Dawleys	Sex	2.155 (1,80)	n.s.
	Stress	0.040 (1,80)	n.s.
Long-Evans	Sex	1.363 (1,76)	n.s.
	Stress	0.237 (1,76)	n.s.

**Table 52. Results of paired t-tests comparing Morris water maze Day 1-Trial 1 times and distances to Day 1-Trial 2 times and distances.**

Strain	Treatment Group	Comparison	t value (d.f.)	p value
Sprague-Dawleys	Male - No Stress	Trial 1 time with Trial 2 Time	3.74 (16)	p = 0.002
		Trial 1 distance with Trial 2 distance	2.68 (14)	p = 0.018
	Male - Stress	Trial 1 time with Trial 2 Time	0.90 (16)	n.s.
		Trial 1 distance with Trial 2 distance	0.10 (16)	n.s.
	Female - No Stress	Trial 1 time with Trial 2 Time	5.43 (16)	p < 0.001
		Trial 1 distance with Trial 2 distance	3.58 (16)	p = 0.003
	Female - Stress	Trial 1 time with Trial 2 Time	3.31 (19)	p = 0.004
		Trial 1 distance with Trial 2 distance	2.58 (19)	p = 0.019
Long-Evans	Male - No Stress	Trial 1 time with Trial 2 Time	4.59 (15)	p < 0.001
		Trial 1 distance with Trial 2 distance	1.63 (11)	n.s.
	Male - Stress	Trial 1 time with Trial 2 Time	3.95 (15)	p = 0.001
		Trial 1 distance with Trial 2 distance	2.45 (11)	p = 0.031
	Female - No Stress	Trial 1 time with Trial 2 Time	3.85 (15)	p = 0.002
		Trial 1 distance with Trial 2 distance	2.51 (15)	p = 0.024
	Female - Stress	Trial 1 time with Trial 2 Time	2.40 (15)	p = 0.030
		Trial 1 distance with Trial 2 distance	1.43 (15)	n.s.

**Table 53. Results of paired t-tests comparing Morris water maze Day 1-Trial 1 times and distances to Day 2-Trial 1 times and distances.**

Strain	Treatment Group	Comparison	t value (d.f.)	p value
Sprague-Dawleys	Male - No Stress	Day 1 - Trial 1 time with Day 2 - Trial 1 time	3.96 (16)	p = 0.001
		Day 1 - Trial 1 distance with Day 2 - Trial 1 distance	2.52 (15)	p = 0.024
	Male - Stress	Day 1 - Trial 1 time with Day 2 - Trial 1 time	5.27 (16)	p < 0.001
		Day 1 - Trial 1 distance with Day 2 - Trial 1 distance	4.50 (15)	p < 0.001
	Female - No Stress	Day 1 - Trial 1 time with Day 2 - Trial 1 time	5.03 (16)	p < 0.001
		Day 1 - Trial 1 distance with Day 2 - Trial 1 distance	3.92 (15)	p = 0.001
	Female - Stress	Day 1 - Trial 1 time with Day 2 - Trial 1 time	6.05 (19)	p < 0.001
		Day 1 - Trial 1 distance with Day 2 - Trial 1 distance	5.81 (18)	p < 0.001
Long-Evans	Male - No Stress	Day 1 - Trial 1 time with Day 2 - Trial 1 time	6.74 (15)	p < 0.001
		Day 1 - Trial 1 distance with Day 2 - Trial 1 distance	4.71 (12)	p = 0.001
	Male - Stress	Day 1 - Trial 1 time with Day 2 - Trial 1 time	7.67 (15)	p < 0.001
		Day 1 - Trial 1 distance with Day 2 - Trial 1 distance	5.07 (12)	p < 0.001
	Female - No Stress	Day 1 - Trial 1 time with Day 2 - Trial 1 time	5.79 (15)	p < 0.001
		Day 1 - Trial 1 distance with Day 2 - Trial 1 distance	4.84 (15)	p < 0.001
	Female - Stress	Day 1 - Trial 1 time with Day 2 - Trial 1 time	4.06 (15)	p = 0.001
		Day 1 - Trial 1 distance with Day 2 - Trial 1 distance	3.29 (15)	p = 0.005

**Table 54. Results of MANOVAs on Morris water maze Day 1 - Trial 1 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 2.56 (2,119) p = 0.082	Day 1 Trial 1 Time	3.95 (1,120)	p = 0.049
		Day 1 Trial 1 Distance	1.77 (1,120)	n.s.
	<b>SEX</b> 0.17 (2,119) n.s.	Day 1 Trial 1 Time	0.02 (1,120)	n.s.
		Day 1 Trial 1 Distance	0.01 (1,120)	n.s.
	<b>STRESS</b> 5.50 (2,119) p = 0.005	Day 1 Trial 1 Time	1.09 (1,120)	n.s.
		Day 1 Trial 1 Distance	0.15 (1,120)	n.s.
	<b>STRAIN X SEX</b> 0.99 (2,119) n.s.	Day 1 Trial 1 Time	1.99 (1,120)	n.s.
		Day 1 Trial 1 Distance	1.63 (1,120)	n.s.
	<b>STRAIN X STRESS</b> 0.34 (2,119) n.s.	Day 1 Trial 1 Time	0.52 (1,120)	n.s.
		Day 1 Trial 1 Distance	0.69 (1,120)	n.s.
Sprague-Dawleys	<b>SEX</b> 0.52 (2,65) n.s.	Day 1 Trial 1 Time	1.73 (1,120)	n.s.
		Day 1 Trial 1 Distance	2.06 (1,120)	n.s.
	<b>STRESS</b> 3.95 (2,65) p = 0.024	Day 1 Trial 1 Time	0.17 (1,120)	n.s.
		Day 1 Trial 1 Distance	1.56 (1,120)	n.s.
	<b>STRAIN X SEX X STRESS</b> 2.16 (2,119) n.s.	Day 1 Trial 1 Time	0.96 (1,66)	n.s.
		Day 1 Trial 1 Distance	1.04 (1,66)	n.s.
	<b>STRESS</b> 3.95 (2,65) p = 0.024	Day 1 Trial 1 Time	1.90 (1,66)	n.s.
		Day 1 Trial 1 Distance	0.10 (1,66)	n.s.
	<b>SEX X STRESS</b> 2.68 (2,65) p = 0.076	Day 1 Trial 1 Time	1.82 (1,66)	n.s.
		Day 1 Trial 1 Distance	3.92 (1,66)	p = 0.052
Long-Evans	<b>SEX</b> 0.51 (2,53) n.s.	Day 1 Trial 1 Time	1.00 (1,54)	n.s.
		Day 1 Trial 1 Distance	0.64 (1,54)	n.s.
	<b>STRESS</b> 2.45 (2,53) p = 0.096	Day 1 Trial 1 Time	0.04 (1,54)	n.s.
		Day 1 Trial 1 Distance	0.70 (1,54)	n.s.
	<b>SEX X STRESS</b> 0.50 (2,53) n.s.	Day 1 Trial 1 Time	0.33 (1,54)	n.s.

		Day 1 Trial 1 Distance	0.01 (1,58)	n.s.
SD Males	<b>STRESS</b> 1.80 (2,30) n.s.	Day 1 Trial 1 Time	3.46 (1,31)	p = 0.072
		Day 1 Trial 1 Distance	2.42 (1,31)	n.s.
SD Females	<b>STRESS</b> 4.48 (2,34) p = 0.019	Day 1 Trial 1 Time	0.00 (1,35)	n.s.
		Day 1 Trial 1 Distance	1.50 (1,35)	n.s.
LE Males	<b>STRESS</b> 2.43 (2,23) n.s.	Day 1 Trial 1 Time	0.31 (1,24)	n.s.
		Day 1 Trial 1 Distance	0.25 (1,24)	n.s.
LE Females	<b>STRESS</b> 0.51 (2,29) n.s.	Day 1 Trial 1 Time	0.07 (1,30)	n.s.
		Day 1 Trial 1 Distance	0.47 (1,30)	n.s.

**Table 55. Results of MANOVAs on Morris water maze Day 1 - Trial 2 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 0.75 (2,122) n.s.	Day 1 Trial 2 Time	1.11 (1,120)	n.s.
		Day 1 Trial 2 Distance	1.36 (1,120)	n.s.
	<b>SEX</b> 0.22 (2,122) n.s.	Day 1 Trial 2 Time	0.21 (1,120)	n.s.
		Day 1 Trial 2 Distance	0.30 (1,120)	n.s.
	<b>STRESS</b> 2.64 (2,122) p = 0.076	Day 1 Trial 2 Time	3.14 (1,120)	p = 0.079
		Day 1 Trial 2 Distance	4.21 (1,120)	p = 0.042
	<b>STRAIN X SEX</b> 0.40 (2,122) n.s.	Day 1 Trial 2 Time	0.21 (1,120)	n.s.
		Day 1 Trial 2 Distance	0.08 (1,120)	n.s.
	<b>STRAIN X STRESS</b> 0.33 (2,122) n.s.	Day 1 Trial 2 Time	0.64 (1,120)	n.s.
		Day 1 Trial 2 Distance	0.57 (1,120)	n.s.
Sprague-Dawleys	<b>SEX</b> 0.53 (2,65) n.s.	Day 1 Trial 2 Time	0.00 (1,66)	n.s.
		Day 1 Trial 2 Distance	0.04 (1,66)	n.s.
	<b>STRESS</b> 2.38 (2,65) n.s.	Day 1 Trial 2 Time	4.36 (1,66)	p = 0.041
		Day 1 Trial 2 Distance	4.78 (1,66)	p = 0.032
	<b>SEX X STRESS</b> 0.61 (2,65) n.s.	Day 1 Trial 2 Time	0.05 (1,66)	n.s.
		Day 1 Trial 2 Distance	0.19 (1,66)	n.s.
	<b>SEX</b> 0.16 (2,53) n.s.	Day 1 Trial 2 Time	0.32 (1,57)	n.s.
		Day 1 Trial 2 Distance	0.28 (1,57)	n.s.
	<b>STRESS</b> 0.78 (2,56) n.s.	Day 1 Trial 2 Time	0.36 (1,57)	n.s.
		Day 1 Trial 2 Distance	0.69 (1,57)	n.s.
Long-Evans	<b>SEX X STRESS</b> 1.90 (2,56) n.s.	Day 1 Trial 2 Time	0.24 (1,57)	n.s.
		Day 1 Trial 2 Distance	0.00 (1,57)	n.s.

Table 56. Results of MANOVAs on Morris water maze Day 2 - Trial 1 times and distances.

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 0.12 (2,122) n.s.	Day 2 Trial 1 Time	0.06 (1,123)	n.s.
		Day 2 Trial 1 Distance	0.13 (1,123)	n.s.
	<b>SEX</b> 0.03 (2,122) n.s.	Day 2 Trial 1 Time	0.00 (1,123)	n.s.
		Day 2 Trial 1 Distance	0.02 (1,123)	n.s.
	<b>STRESS</b> 1.03 (2,122) n.s.	Day 2 Trial 1 Time	1.86 (1,123)	n.s.
		Day 2 Trial 1 Distance	1.39 (1,123)	n.s.
	<b>STRAIN X SEX</b> 0.80 (2,122) n.s.	Day 2 Trial 1 Time	0.01 (1,123)	n.s.
		Day 2 Trial 1 Distance	0.05 (1,123)	n.s.
	<b>STRAIN X STRESS</b> 4.90 (2,122) p = 0.009	Day 2 Trial 1 Time	1.98 (1,123)	n.s.
		Day 2 Trial 1 Distance	4.59 (1,123)	p = 0.034
Sprague-Dawleys	<b>SEX</b> 0.39 (2,62) n.s.	Day 2 Trial 1 Time	6.12 (1,123)	p = 0.015
		Day 2 Trial 1 Distance	5.96 (1,123)	p = 0.016
	<b>STRESS</b> 3.51 (2,62) p = 0.036	Day 2 Trial 1 Time	0.14 (1,123)	n.s.
		Day 2 Trial 1 Distance	0.04 (1,123)	n.s.
	<b>SEX X STRESS</b> 2.50 (2,62) p = 0.091	Day 2 Trial 1 Time	2.67 (1,63)	n.s.
		Day 2 Trial 1 Distance	3.90 (1,63)	p = 0.053
	<b>SEX</b> 0.44 (2,59) n.s.	Day 2 Trial 1 Time	0.00 (1,60)	n.s.
		Day 2 Trial 1 Distance	0.07 (1,60)	n.s.
	<b>STRESS</b> 2.11 (2,59) n.s.	Day 2 Trial 1 Time	0.00 (1,60)	n.s.
		Day 2 Trial 1 Distance	0.41 (1,60)	n.s.
Long-Evans	<b>SEX X STRESS</b> 2.05 (2,59) n.s.	Day 2 Trial 1 Time	3.41 (1,60)	p = 0.070
		Day 2 Trial 1 Distance	2.26 (1,60)	n.s.
	<b>STRESS</b> 6.00 (2,29) p = 0.007	Day 2 Trial 1 Time	6.03 (1,30)	p = 0.020
		Day 2 Trial 1 Distance	8.27 (1,30)	p = 0.007
	<b>STRESS</b> 0.08 (2,32) n.s.	Day 2 Trial 1 Time	0.17 (1,33)	n.s.
		Day 2 Trial 1 Distance	0.16 (1,33)	n.s.

**Table 57. Results of MANOVAs on Morris water maze Day 2 - Trial 2 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 7.51 (2,126) p = 0.001	Day 2 Trial 2 Time	4.67 (1,127)	p = 0.032
		Day 2 Trial 2 Distance	7.54 (1,127)	p = 0.015
	<b>SEX</b> 2.34 (2,126) n.s.	Day 2 Trial 2 Time	4.11 (1,127)	p = 0.045
		Day 2 Trial 2 Distance	3.39 (1,127)	p = 0.068
	<b>STRESS</b> 0.77 (2,126) n.s.	Day 2 Trial 2 Time	0.15 (1,127)	n.s.
		Day 2 Trial 2 Distance	0.02 (1,127)	n.s.
	<b>STRAIN X SEX</b> 1.17 (2,126) n.s.	Day 2 Trial 2 Time	0.63 (1,127)	n.s.
		Day 2 Trial 2 Distance	1.07 (1,127)	n.s.
	<b>STRAIN X STRESS</b> 0.82 (2,126) n.s.	Day 2 Trial 2 Time	1.44 (1,127)	n.s.
		Day 2 Trial 2 Distance	1.61 (1,127)	n.s.
Males	<b>STRESS</b> 0.05 (2,126) n.s.	Day 2 Trial 2 Time	0.10 (1,127)	n.s.
		Day 2 Trial 2 Distance	0.08 (1,127)	n.s.
	<b>STRAIN X SEX X STRESS</b> 0.29 (2,126) n.s.	Day 2 Trial 2 Time	0.25 (1,127)	n.s.
		Day 2 Trial 2 Distance	0.14 (1,127)	n.s.
	<b>STRAIN</b> 1.60 (2,61) n.s.	Day 2 Trial 2 Time	1.02 (1,62)	n.s.
		Day 2 Trial 2 Distance	1.53 (1,62)	n.s.
	<b>STRESS</b> 0.84 (2,61) n.s.	Day 2 Trial 2 Time	0.26 (1,62)	n.s.
		Day 2 Trial 2 Distance	0.10 (1,62)	n.s.
	<b>STRAIN X STRESS</b> 0.47 (2,61) n.s.	Day 2 Trial 2 Time	0.27 (1,62)	n.s.
		Day 2 Trial 2 Distance	0.42 (1,62)	n.s.
Females	<b>STRAIN</b> 6.58 (2,64) p = 0.003	Day 2 Trial 2 Time	4.08 (1,65)	p = 0.048
		Day 2 Trial 2 Distance	6.90 (1,65)	p = 0.011.
	<b>STRESS</b> 0.22 (2,64) n.s.	Day 2 Trial 2 Time	0.00 (1,65)	n.s.
		Day 2 Trial 2 Distance	0.01 (1,65)	n.s.
	<b>STRAIN X STRESS</b> 0.66 (2,64) n.s.	Day 2 Trial 2 Time	1.34 (1,65)	n.s.
		Day 2 Trial 2 Distance	1.30 (1,65)	n.s.

**Table 58.** Results of paired t-tests comparing Morris water maze averaged Trial 1 times and distances (from Days 3 through 7) to averaged Trial 2 times and distances (from Days 3 through 7).

Strain	Treatment Group	Comparison	t value (d.f.)	p value
Sprague-Dawleys	Male - No Stress	Average Trial 1 time with average Trial 2 time	2.86 (16)	p = 0.011
		Average Trial 1 distance average Trial 2 distance	2.52 (16)	p = 0.023
	Male - Stress	Average Trial 1 time with average Trial 2 time	4.73 (16)	p < 0.001
		Average Trial 1 distance average Trial 2 distance	4.27 (16)	p < 0.001
	Female - No Stress	Average Trial 1 time with average Trial 2 time	2.77 (16)	p = 0.014
		Average Trial 1 distance average Trial 2 distance	1.89 (16)	p = 0.078
	Female - Stress	Average Trial 1 time with average Trial 2 time	4.88 (19)	p < 0.001
		Average Trial 1 distance average Trial 2 distance	5.01 (19)	p < 0.001
Long-Evans	Male - No Stress	Average Trial 1 time with average Trial 2 time	7.83 (15)	p < 0.001
		Average Trial 1 distance average Trial 2 distance	5.93 (15)	p < 0.001
	Male - Stress	Average Trial 1 time with average Trial 2 time	5.60 (15)	p < 0.001
		Average Trial 1 distance average Trial 2 distance	3.62 (15)	p = 0.003
	Female - No Stress	Average Trial 1 time with average Trial 2 time	3.45 (15)	p = 0.004
		Average Trial 1 distance average Trial 2 distance	3.27 (15)	p = 0.005
	Female - Stress	Average Trial 1 time with average Trial 2 time	5.60 (15)	p < 0.001
		Average Trial 1 distance average Trial 2 distance	6.10 (15)	p < 0.001

Table 59. Results of MANOVAs on Morris water maze Day 3 - Trial 1 times and distances.

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 1.31 (2,103) n.s.	Day 3 Trial 1 Time	0.25 (1,104)	n.s.
		Day 3 Trial 1 Distance	0.04 (1,104)	n.s.
	<b>SEX</b> 2.67 (2,103) p = 0.074	Day 3 Trial 1 Time	4.13 (1,104)	p = 0.045
		Day 3 Trial 1 Distance	1.89 (1,104)	n.s.
	<b>STRESS</b> 3.21 (2,103) p = 0.045	Day 3 Trial 1 Time	1.84 (1,104)	n.s.
		Day 3 Trial 1 Distance	4.56 (1,104)	p = 0.035
	<b>STRAIN X SEX</b> 1.26 (2,103) n.s.	Day 3 Trial 1 Time	0.02 (1,104)	n.s.
		Day 3 Trial 1 Distance	0.30 (1,104)	n.s.
	<b>STRAIN X STRESS</b> 0.48 (2,103) n.s.	Day 3 Trial 1 Time	0.17 (1,104)	n.s.
		Day 3 Trial 1 Distance	0.00 (1,104)	n.s.
Sprague-Dawleys	<b>SEX</b> 4.39 (2,52) p = 0.017	Day 3 Trial 1 Time	2.13 (1,53)	n.s.
		Day 3 Trial 1 Distance	0.28 (1,53)	n.s.
	<b>STRESS</b> 3.15 (2,52) p = 0.051	Day 3 Trial 1 Time	0.41 (1,53)	n.s.
		Day 3 Trial 1 Distance	1.95 (1,53)	n.s.
	<b>SEX X STRESS</b> 0.60 (2,52) n.s.	Day 3 Trial 1 Time	0.23 (1,53)	n.s.
		Day 3 Trial 1 Distance	0.61 (1,53)	n.s.
	<b>SEX</b> 1.16 (2,50) n.s.	Day 3 Trial 1 Time	2.04 (1,51)	n.s.
		Day 3 Trial 1 Distance	2.29 (1,51)	n.s.
	<b>STRESS</b> 1.40 (2,50) n.s.	Day 3 Trial 1 Time	1.75 (1,51)	n.s.
		Day 3 Trial 1 Distance	2.81 (1,51)	n.s.
Long-Evans	<b>SEX X STRESS</b> 3.35 (2,50) p = 0.043	Day 3 Trial 1 Time	5.59 (1,51)	p = 0.022
		Day 3 Trial 1 Distance	6.75 (1,51)	P = 0.012
	<b>SD Males</b>	Day 3 Trial 1 Time	0.66 (1,27)	n.s.
		Day 3 Trial 1 Distance	2.27 (1,27)	n.s.
	<b>SD Females</b>	Day 3 Trial 1 Time	0.01 (1,26)	n.s.
		Day 3 Trial 1 Distance	0.20 (1,26)	n.s.
	<b>LE Males</b>	Day 3 Trial 1 Time	0.52 (1,27)	n.s.
		Day 3 Trial 1 Distance	0.43 (1,27)	n.s.
	<b>LE Females</b>	Day 3 Trial 1 Time	7.34 (1,24)	p = 0.012
		Day 3 Trial 1 Distance	9.10 (1,24)	p = 0.006

**Table 60. Results of MANOVAs on Morris water maze Day 3 - Trial 2 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 1.87 (2,104) n.s.	Day 3 Trial 2 Time	2.38 (1,105)	n.s.
		Day 3 Trial 2 Distance	1.38 (1,105)	n.s.
	<b>SEX</b> 9.97 (2,104) p < 0.001	Day 3 Trial 2 Time	10.57 (1,105)	p = 0.002
		Day 3 Trial 2 Distance	5.27 (1,105)	p = 0.024
	<b>STRESS</b> 0.04 (2,104) n.s.	Day 3 Trial 2 Time	0.00 (1,105)	n.s.
		Day 3 Trial 2 Distance	0.01 (1,105)	n.s.
	<b>STRAIN X SEX</b> 1.03 (2,104) n.s.	Day 3 Trial 2 Time	1.74 (1,105)	n.s.
		Day 3 Trial 2 Distance	1.24 (1,105)	n.s.
	<b>STRAIN X STRESS</b> 0.29 (2,104) n.s.	Day 3 Trial 2 Time	0.45 (1,105)	n.s.
		Day 3 Trial 2 Distance	0.56 (1,105)	n.s.
Sprague-Dawleys	<b>SEX</b> 5.36 (2,53) p = 0.008	Day 3 Trial 2 Time	2.07 (1,54)	n.s.
		Day 3 Trial 2 Distance	0.65 (1,54)	n.s.
	<b>STRESS</b> 0.19 (2,53) n.s.	Day 3 Trial 2 Time	0.27 (1,54)	n.s.
		Day 3 Trial 2 Distance	0.33 (1,54)	n.s.
	<b>SEX X STRESS</b> 0.25 (2,53) n.s.	Day 3 Trial 2 Time	0.47 (1,54)	n.s.
		Day 3 Trial 2 Distance	0.50 (1,54)	n.s.
Long-Evans	<b>SEX</b> 5.51 (2,50) p = 0.007	Day 3 Trial 2 Time	9.41 (1,51)	p = 0.003
		Day 3 Trial 2 Distance	6.33 (1,51)	p = 0.015
	<b>STRESS</b> 0.11 (2,50) n.s.	Day 3 Trial 2 Time	0.18 (1,51)	n.s.
		Day 3 Trial 2 Distance	0.22 (1,51)	n.s.
	<b>SEX X STRESS</b> 0.13 (2,50) n.s.	Day 3 Trial 2 Time	0.10 (1,51)	n.s.
		Day 3 Trial 2 Distance	0.18 (1,51)	n.s.

**Table 61. Results of MANOVAs on Morris water maze Day 4 - Trial 1 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 0.74 (2,109) n.s.	Day 4 Trial 1 Time	0.62 (1,110)	n.s.
		Day 4 Trial 1 Distance	0.98 (1,110)	n.s.
	<b>SEX</b> 0.08 (2,109) n.s.	Day 4 Trial 1 Time	0.05 (1,110)	n.s.
		Day 4 Trial 1 Distance	0.01 (1,110)	n.s.
	<b>STRESS</b> 4.47 (2,109) p = 0.014	Day 4 Trial 1 Time	0.05 (1,110)	n.s.
		Day 4 Trial 1 Distance	0.26 (1,110)	n.s.
	<b>STRAIN X SEX</b> 0.56 (2,109) n.s.	Day 4 Trial 1 Time	0.26 (1,110)	n.s.
		Day 4 Trial 1 Distance	0.07 (1,110)	n.s.
	<b>STRAIN X STRESS</b> 3.63 (2,109) p = 0.030	Day 4 Trial 1 Time	3.48 (1,110)	p = 0.065
		Day 4 Trial 1 Distance	1.78 (1,110)	n.s.
Sprague-Dawleys	<b>SEX</b> 0.18 (2,61) n.s.	Day 4 Trial 1 Time	0.04 (1,62)	n.s.
		Day 4 Trial 1 Distance	0.00 (1,62)	n.s.
	<b>STRESS</b> 1.21 (2,61) n.s.	Day 4 Trial 1 Time	1.52 (1,62)	n.s.
		Day 4 Trial 1 Distance	1.97 (1,62)	n.s.
	<b>SEX X STRESS</b> 0.73 (2,61) n.s.	Day 4 Trial 1 Time	0.03 (1,62)	n.s.
		Day 4 Trial 1 Distance	0.16 (1,62)	n.s.
	<b>SEX</b> 0.36 (2,47) n.s.	Day 4 Trial 1 Time	0.24 (1,48)	n.s.
		Day 4 Trial 1 Distance	0.07 (1,48)	n.s.
	<b>STRESS</b> 4.76 (2,47) p = 0.013	Day 4 Trial 1 Time	1.99 (1,48)	n.s.
		Day 4 Trial 1 Distance	0.29 (1,48)	n.s.
Long-Evans	<b>SEX X STRESS</b> 1.50 (2,47) n.s.	Day 4 Trial 1 Time	1.50 (1,48)	n.s.
		Day 4 Trial 1 Distance	0.66 (1,48)	n.s.
	<b>STRESS</b> 5.47 (2,28) p = 0.010	Day 4 Trial 1 Time	3.74 (1,29)	p = 0.063
		Day 4 Trial 1 Distance	1.04 (1,29)	n.s.
LE Males	<b>STRESS</b> 0.73 (2,18) n.s.	Day 4 Trial 1 Time	0.02 (1,19)	n.s.
		Day 4 Trial 1 Distance	0.04 (1,19)	n.s.
LE Females	<b>STRESS</b> 0.73 (2,18) n.s.	Day 4 Trial 1 Time	0.02 (1,19)	n.s.
		Day 4 Trial 1 Distance	0.04 (1,19)	n.s.

Table 62. Results of MANOVAs on Morris water maze Day 4 - Trial 2 times and distances.

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 3.93 (2,111) p = 0.022	Day 4 Trial 2 Time	0.28 (1,112)	n.s.
		Day 4 Trial 2 Distance	1.32 (1,112)	n.s.
	<b>SEX</b> 2.34 (2,111) n.s.	Day 4 Trial 2 Time	4.67 (1,112)	p = 0.033
		Day 4 Trial 2 Distance	4.66 (1,112)	p = 0.033
	<b>STRESS</b> 0.94 (2,111) n.s.	Day 4 Trial 2 Time	0.04 (1,112)	n.s.
		Day 4 Trial 2 Distance	0.25 (1,112)	n.s.
	<b>STRAIN X SEX</b> 0.91 (2,111) n.s.	Day 4 Trial 2 Time	0.99 (1,112)	n.s.
		Day 4 Trial 2 Distance	0.57 (1,112)	n.s.
	<b>STRAIN X STRESS</b> 0.75 (2,111) n.s.	Day 4 Trial 2 Time	0.09 (1,112)	n.s.
		Day 4 Trial 2 Distance	0.00 (1,112)	n.s.
Sprague-Dawleys	<b>SEX</b> 2.91 (2,62) p = 0.062	Day 4 Trial 2 Time	5.90 (1,63)	p = 0.018
		Day 4 Trial 2 Distance	5.44 (1,63)	p = 0.023
	<b>STRESS</b> 1.53 (2,62) n.s.	Day 4 Trial 2 Time	0.00 (1,63)	n.s.
		Day 4 Trial 2 Distance	0.15 (1,63)	n.s.
	<b>SEX X STRESS</b> 3.36 (2,62) p = 0.041	Day 4 Trial 2 Time	0.65 (1,63)	n.s.
		Day 4 Trial 2 Distance	0.01 (1,63)	n.s.
	<b>SEX</b> 0.54 (2,48) n.s.	Day 4 Trial 2 Time	0.58 (1,49)	n.s.
		Day 4 Trial 2 Distance	0.77 (1,49)	n.s.
	<b>STRESS</b> 0.05 (2,48) n.s.	Day 4 Trial 2 Time	0.10 (1,49)	n.s.
		Day 4 Trial 2 Distance	0.10 (1,49)	n.s.
Long-Evans	<b>SEX X STRESS</b> 0.57 (2,48) n.s.	Day 4 Trial 2 Time	0.09 (1,49)	n.s.
		Day 4 Trial 2 Distance	0.02 (1,49)	n.s.

**Table 63. Results of MANOVAs on Morris water maze Day 5 - Trial 1 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 2.00 (2,109) n.s.	Day 5 Trial 1 Time	0.03 (1,110)	n.s.
		Day 5 Trial 1 Distance	0.72 (1,110)	n.s.
	<b>SEX</b> 0.04 (2,109) n.s.	Day 5 Trial 1 Time	0.07 (1,110)	n.s.
		Day 5 Trial 1 Distance	0.06 (1,110)	n.s.
	<b>STRESS</b> 1.75 (2,109) n.s.	Day 5 Trial 1 Time	0.17 (1,110)	n.s.
		Day 5 Trial 1 Distance	0.06 (1,110)	n.s.
	<b>STRAIN X SEX</b> 0.38 (2,109) n.s.	Day 5 Trial 1 Time	0.00 (1,110)	n.s.
		Day 5 Trial 1 Distance	0.10 (1,110)	n.s.
	<b>STRAIN X STRESS</b> 0.24 (2,109) n.s.	Day 5 Trial 1 Time	0.25 (1,110)	n.s.
		Day 5 Trial 1 Distance	0.09 (1,110)	n.s.
	<b>SEX X STRESS</b> 1.41 (2,109) n.s.	Day 5 Trial 1 Time	1.53 (1,110)	n.s.
		Day 5 Trial 1 Distance	0.58 (1,110)	n.s.
	<b>STRAIN X SEX X STRESS</b> 0.61 (2,109) n.s.	Day 5 Trial 1 Time	0.26 (1,110)	n.s.
		Day 5 Trial 1 Distance	0.68 (1,110)	n.s.

**Table 64. Results of MANOVAs on Morris water maze Day 5 - Trial 2 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 1.91 (2,110) n.s.	Day 5 Trial 2 Time	3.84 (1,111)	p = 0.053
		Day 5 Trial 2 Distance	3.75 (1,111)	p = 0.055
	<b>SEX</b> 4.15 (2,110) p = 0.018	Day 5 Trial 2 Time	6.33 (1,111)	p = 0.013
		Day 5 Trial 2 Distance	4.21 (1,111)	p = 0.043
	<b>STRESS</b> 3.45 (2,110) p = 0.035	Day 5 Trial 2 Time	0.49 (1,111)	n.s.
		Day 5 Trial 2 Distance	0.00 (1,111)	n.s.
	<b>STRAIN X SEX</b> 0.06 (2,110) n.s.	Day 5 Trial 2 Time	0.00 (1,111)	n.s.
		Day 5 Trial 2 Distance	0.01 (1,111)	n.s.
	<b>STRAIN X STRESS</b> 0.72 (2,110) n.s.	Day 5 Trial 2 Time	1.32 (1,111)	n.s.
		Day 5 Trial 2 Distance	1.02 (1,111)	n.s.
Sprague-Dawleys	<b>SEX</b> 3.11 (2,59) p = 0.052	Day 5 Trial 2 Time	4.47 (1,60)	p = 0.039
		Day 5 Trial 2 Distance	3.04 (1,60)	p = 0.086
	<b>STRESS</b> 1.94 (2,59) n.s.	Day 5 Trial 2 Time	0.14 (1,60)	n.s.
		Day 5 Trial 2 Distance	0.66 (1,60)	n.s.
	<b>SEX X STRESS</b> 0.48 (2,59) n.s.	Day 5 Trial 2 Time	0.56 (1,60)	n.s.
		Day 5 Trial 2 Distance	0.33 (1,60)	n.s.
	<b>SEX</b> 1.48 (2,50) n.s.	Day 5 Trial 2 Time	2.30 (1,51)	n.s.
		Day 5 Trial 2 Distance	1.47 (1,51)	n.s.
	<b>STRESS</b> 1.80 (2,50) n.s.	Day 5 Trial 2 Time	1.25 (1,51)	n.s.
		Day 5 Trial 2 Distance	0.40 (1,51)	n.s.
Long-Evans	<b>SEX X STRESS</b> 0.26 (2,50) n.s.	Day 5 Trial 2 Time	0.51 (1,51)	n.s.
		Day 5 Trial 2 Distance	0.54 (1,51)	n.s.

**Table 65. Results of MANOVAs on Morris water maze Day 6 - Trial 1 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 7.73 (2,105) p = 0.001	Day 6 Trial 1 Time	9.83 (1,106)	p = 0.002
		Day 6 Trial 1 Distance	4.33 (1,106)	p = 0.040
	<b>SEX</b> 3.37 (2,105) p = 0.038	Day 6 Trial 1 Time	4.03 (1,106)	p = 0.047
		Day 6 Trial 1 Distance	1.66 (1,106)	n.s.
	<b>STRESS</b> 12.85 (2,105) p < 0.001	Day 6 Trial 1 Time	8.33 (1,106)	p = 0.005
		Day 6 Trial 1 Distance	1.47 (1,106)	n.s.
	<b>STRAIN X SEX</b> 0.32 (2,105) n.s.	Day 6 Trial 1 Time	0.16 (1,106)	n.s.
		Day 6 Trial 1 Distance	0.02 (1,106)	n.s.
	<b>STRAIN X STRESS</b> 2.35 (2,105) n.s.	Day 6 Trial 1 Time	0.32 (1,106)	n.s.
		Day 6 Trial 1 Distance	0.05 (1,106)	n.s.
Sprague-Dawleys	<b>SEX</b> 1.92 (2,59) n.s.	Day 6 Trial 1 Time	1.89 (1,60)	n.s.
		Day 6 Trial 1 Distance	0.94 (1,60)	n.s.
	<b>STRESS</b> 5.96 (2,59) p = 0.004	Day 6 Trial 1 Time	3.93 (1,60)	n.s.
		Day 6 Trial 1 Distance	1.42 (1,60)	n.s.
	<b>SEX X STRESS</b> 2.34 (2,59) n.s.	Day 6 Trial 1 Time	3.42 (1,60)	n.s.
		Day 6 Trial 1 Distance	4.34 (1,60)	n.s.
	<b>SEX</b> 1.50 (2,45) n.s.	Day 6 Trial 1 Time	2.01 (1,46)	n.s.
		Day 6 Trial 1 Distance	0.73 (1,46)	n.s.
	<b>STRESS</b> 6.26 (2,45) p = 0.004	Day 6 Trial 1 Time	4.14 (1,46)	n.s.
		Day 6 Trial 1 Distance	0.36 (1,46)	n.s.
Long-Evans	<b>SEX X STRESS</b> 1.76 (2,45) n.s.	Day 6 Trial 1 Time	0.67 (1,46)	p = 0.022
		Day 6 Trial 1 Distance	2.14 (1,46)	p = 0.012
	<b>SD Males</b>	Day 6 Trial 1 Time	11.21 (1,32)	p = 0.002
		Day 6 Trial 1 Distance	7.09 (1,32)	p = 0.012
	<b>SD Females</b>	Day 6 Trial 1 Time	0.00 (1,28)	n.s.
		Day 6 Trial 1 Distance	0.31 (1,28)	n.s.

<b>LE Males</b>	<b>STRESS</b> 3.58 (2,28) $p = 0.041$	<b>Day 6 Trial 1 Time</b>	<b>5.88 (1,29)</b>	<b>p = 0.022</b>
		<b>Day 6 Trial 1 Distance</b>	<b>2.81 (1,29)</b>	<b>n.s.</b>
<b>LE Females</b>	<b>STRESS</b> 4.42 (2,16) $p = 0.030$	<b>Day 6 Trial 1 Time</b>	<b>0.54 (1,17)</b>	<b>n.s.</b>
		<b>Day 6 Trial 1 Distance</b>	<b>0.32 (1,17)</b>	<b>n.s.</b>

Table 66. Results of MANOVAs on Morris water maze Day 6 - Trial 2 times and distances.

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 0.33 (2,105) n.s.	Day 6 Trial 2 Time	0.55 (1,106)	n.s.
		Day 6 Trial 2 Distance	0.41 (1,106)	n.s.
	<b>SEX</b> 9.59 (2,105) $p < 0.001$	Day 6 Trial 2 Time	0.72 (1,106)	n.s.
		Day 6 Trial 2 Distance	0.08 (1,106)	n.s.
	<b>STRESS</b> 6.52 (2,105) $p = 0.002$	Day 6 Trial 2 Time	0.07 (1,106)	n.s.
		Day 6 Trial 2 Distance	0.43 (1,106)	n.s.
	<b>STRAIN X SEX</b> 4.52 (2,105) $p = 0.013$	Day 6 Trial 2 Time	0.43 (1,106)	n.s.
		Day 6 Trial 2 Distance	1.91 (1,106)	n.s.
	<b>STRAIN X STRESS</b> 4.28 (2,105) $p = 0.016$	Day 6 Trial 2 Time	0.02 (1,106)	n.s.
		Day 6 Trial 2 Distance	0.39 (1,106)	n.s.
	<b>SEX X STRESS</b> 2.16 (2,105) n.s.	Day 6 Trial 2 Time	0.64 (1,106)	n.s.
		Day 6 Trial 2 Distance	0.08 (1,106)	n.s.
	<b>STRAIN X SEX X STRESS</b> 4.02 (2,105) $p = 0.021$	Day 6 Trial 2 Time	2.21 (1,106)	n.s.
		Day 6 Trial 2 Distance	0.67 (1,106)	n.s.
Sprague-Dawleys	<b>SEX</b> 2.29 (2,60) n.s.	Day 6 Trial 2 Time	1.48 (1,61)	n.s.
		Day 6 Trial 2 Distance	0.82 (1,61)	n.s.
	<b>STRESS</b> 0.37 (2,60) n.s.	Day 6 Trial 2 Time	0.01 (1,61)	n.s.
		Day 6 Trial 2 Distance	0.00 (1,61)	n.s.
	<b>SEX X STRESS</b> 0.37 (2,60) n.s.	Day 6 Trial 2 Time	0.31 (1,61)	n.s.
		Day 6 Trial 2 Distance	0.19 (1,61)	n.s.
Long-Evans	<b>SEX</b> 5.89 (2,44) n.s.	Day 6 Trial 2 Time	0.02 (1,45)	n.s.
		Day 6 Trial 2 Distance	1.06 (1,45)	n.s.
	<b>STRESS</b> 4.80 (2,44) $p = 0.013$	Day 6 Trial 2 Time	0.06 (1,45)	n.s.
		Day 6 Trial 2 Distance	0.62 (1,45)	n.s.
	<b>SEX X STRESS</b> 3.04 (2,44) $p = 0.058$	Day 6 Trial 2 Time	2.06 (1,45)	n.s.
		Day 6 Trial 2 Distance	0.47 (1,45)	n.s.
LE Males	<b>STRESS</b> 0.65 (2,28) n.s.	Day 6 Trial 2 Time	1.02 (1,29)	n.s.
		Day 6 Trial 2 Distance	1.22 (1,29)	n.s.
LE Females	<b>STRESS</b> 4.3 (2,15) $p = 0.026$	Day 6 Trial 2 Time	1.09 (1,16)	n.s.
		Day 6 Trial 2 Distance	0.01 (1,16)	n.s.

**Table 67 Results of MANOVAs on Morris water maze Day 7- Trial 1 times and distances.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 8.24 (2,104) p < 0.001	Day 7 Trial 1 Time	12.69 (1,105)	p = 0.001
		Day 7 Trial 1 Distance	7.95 (1,105)	p = 0.006
	<b>SEX</b> 5.67 (2,104) p = 0.005	Day 7 Trial 1 Time	11.41 (1,105)	p = 0.001
		Day 7 Trial 1 Distance	10.83 (1,105)	p = 0.001
	<b>STRESS</b> 5.15 (2,104) p = 0.007	Day 7 Trial 1 Time	3.18 (1,105)	p = 0.078
		Day 7 Trial 1 Distance	0.83 (1,105)	n.s.
	<b>STRAIN X SEX</b> 0.15 (2,104) n.s.	Day 7 Trial 1 Time	0.29 (1,105)	n.s.
		Day 7 Trial 1 Distance	0.25 (1,105)	n.s.
	<b>STRAIN X STRESS</b> 6.90 (2,104) p = 0.002	Day 7 Trial 1 Time	13.84 (1,105)	p < 0.001
		Day 7 Trial 1 Distance	12.02 (1,105)	p = 0.001
Sprague-Dawleys	<b>SEX</b> 3.00 (2,58) p = 0.058	Day 7 Trial 1 Time	6.05 (1,59)	p = 0.017
		Day 7 Trial 1 Distance	5.34 (1,59)	p = 0.024
	<b>STRESS</b> 3.12 (2,58) p = 0.052	Day 7 Trial 1 Time	2.82 (1,59)	p = 0.098
		Day 7 Trial 1 Distance	4.47 (1,59)	p = 0.039
	<b>SEX X STRESS</b> 0.57 (2,58) n.s.	Day 7 Trial 1 Time	1.12 (1,59)	n.s.
Long-Evans	<b>SEX</b> 2.63 (2,45) p = 0.083	Day 7 Trial 1 Time	5.22 (1,46)	p = 0.027
		Day 7 Trial 1 Distance	5.27 (1,46)	p = 0.026
	<b>STRESS</b> 5.81 (2,45) p = 0.006	Day 7 Trial 1 Time	10.29 (1,46)	p = 0.002
		Day 7 Trial 1 Distance	7.03 (1,46)	p = 0.011
	<b>SEX X STRESS</b> 3.65 (2,45) p = 0.034	Day 7 Trial 1 Time	4.04 (1,46)	p = 0.050
SD Males	<b>STRESS</b> 2.23 (2,30) n.s.	Day 7 Trial 1 Distance	1.77 (1,46)	n.s.
		Day 7 Trial 1 Time	0.33 (1,31)	n.s.
SD Females	<b>STRESS</b> 1.98 (2,27) n.s.	Day 7 Trial 1 Distance	0.94 (1,31)	n.s.
		Day 7 Trial 1 Time	2.50 (1,28)	n.s.
		Day 7 Trial 1 Distance	3.51 (1,28)	p = 0.072

<b>LE Males</b>	<b>STRESS</b> 0.68 (2,27) n.s.	Day 7 Trial 1 Time	0.83 (1,28)	n.s.
		Day 7 Trial 1 Distance	1.12 (1,28)	n.s.
<b>LE Females</b>	<b>STRESS</b> 7.70 (2,17) p = 0.004	Day 7 Trial 1 Time	12.98 (1,18)	p = 0.002
		Day 7 Trial 1 Distance	6.41 (1,18)	p = 0.021

Table 68. Results of MANOVAs on Morris water maze Day 7 - Trial 2 times and distances.

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 3.86 (2,102) p = 0.024	Day 7 Trial 2 Time	5.66 (1,103)	p = 0.019
		Day 7 Trial 2 Distance	2.37 (1,103)	n.s.
	<b>SEX</b> 4.93 (2,102) p = 0.009	Day 7 Trial 2 Time	8.52 (1,103)	p = 0.004
		Day 7 Trial 2 Distance	4.58 (1,103)	p = 0.035
	<b>STRESS</b> 0.47 (2,102) n.s.	Day 7 Trial 2 Time	0.96 (1,103)	n.s.
		Day 7 Trial 2 Distance	0.77 (1,103)	n.s.
	<b>STRAIN X SEX</b> 1.07 (2,102) n.s.	Day 7 Trial 2 Time	1.44 (1,103)	n.s.
		Day 7 Trial 2 Distance	0.53 (1,103)	n.s.
	<b>STRAIN X STRESS</b> 1.15 (2,102) n.s.	Day 7 Trial 2 Time	1.27 (1,103)	n.s.
		Day 7 Trial 2 Distance	0.35 (1,103)	n.s.
Sprague-Dawleys	<b>SEX</b> 1.20 (2,58) n.s.	Day 7 Trial 2 Time	2.17 (1,59)	n.s.
		Day 7 Trial 2 Distance	1.30 (1,59)	n.s.
	<b>STRESS</b> 0.37 (2,58) n.s.	Day 7 Trial 2 Time	0.02 (1,59)	n.s.
		Day 7 Trial 2 Distance	0.05 (1,59)	n.s.
	<b>SEX X STRESS</b> 2.24 (2,58) n.s.	Day 7 Trial 2 Time	0.13 (1,59)	n.s.
		Day 7 Trial 2 Distance	0.27 (1,59)	n.s.
	<b>SEX</b> 3.25 (2,43) p = 0.049	Day 7 Trial 2 Time	5.83 (1,44)	p = 0.020
		Day 7 Trial 2 Distance	3.18 (1,44)	p = 0.081
	<b>STRESS</b> 0.85 (2,43) n.s.	Day 7 Trial 2 Time	1.52 (1,44)	n.s.
		Day 7 Trial 2 Distance	0.84 (1,44)	n.s.
Long-Evans	<b>SEX X STRESS</b> 0.10 (2,43) n.s.	Day 7 Trial 2 Time	0.00 (1,44)	n.s.
		Day 7 Trial 2 Distance	0.05 (1,44)	n.s.

**Table 69: Results of chi-squares on number of animals that sat on the water maze platform for at least 20 sec vs. those that did not sit for at least 20 sec on each day.**

<b>Group Tested</b>	<b>Day 3</b>	<b>Day 4</b>	<b>Day 5</b>	<b>Day 6</b>	<b>Day 7</b>
All animals	64.07 (p<0.001)	81.67 (p<0.001)	78.59 (p<0.001)	72.60 (p<0.001)	61.34 (p<0.001)
Sprague-Dawley	31.13 (p<0.001)	55.90 (p<0.001)	45.76 (p<0.001)	52.41 (p<0.001)	42.61 (p<0.001)
Long-Evans	33.06 (p<0.001)	27.56 (p<0.001)	33.06 (p<0.001)	22.56 (p<0.001)	20.25 (p<0.001)
Males	40.97 (p<0.001)	54.55 (p<0.001)	50.97 (p<0.001)	All sat	54.55 (p<0.001)
Females	24.36 (p<0.001)	29.35 (p<0.001)	29.35 (p<0.001)	15.78 (p<0.001)	13.93 (p<0.001)
SD males	19.88 (p<0.001)	26.47 (p<0.001)	23.06 (p<0.001)	All sat	30.12 (p<0.001)
SD females	11.92 (p=0.001)	29.43 (p<0.001)	22.73 (p<0.001)	19.70 (p<0.001)	14.30 (p<0.001)
LE males	21.13 (p<0.001)	28.13 (p<0.001)	28.13 (p<0.001)	All sat	24.50 (p<0.001)
LE females	12.50 (p<0.001)	4.50 (p=0.034)	8.00 (p=0.005)	1.13 n.s.	2.00 n.s.
SD males-No Stress	9.94 (p=0.002)	9.94 (p=0.002)	9.94 (p=0.002)	All sat	13.24 (p<0.001)
SD males-Stress	9.94 (p=0.002)	All sat	13.24 (p<0.001)	All sat	All sat
SD females-No Stress	7.12 (p=0.008)	13.24 (p<0.001)	9.94 (p=0.002)	13.24 (p<0.001)	7.12 (p=0.008)
SD females-Stress	5.00 (p=0.025)	16.20 (p<0.001)	12.80 (p<0.001)	7.2 (p=0.007)	7.2 (p=0.007)
LE males-No Stress	9.00 (p=0.003)	12.25 (p<0.001)	All sat	All sat	All sat
LE males-Stress	12.25 (p<0.001)	All sat	12.25 (p<0.001)	All sat	9.00 (p=0.003)

LE females-No Stress	2.25 n.s.	1.00 n.s.	0.25 n.s.	0.25 n.s.	0.25 n.s.
LE females-Stress	12.25 (p<0.001)	4.00 (p=0.046)	12.25 (p<0.001)	4.00 (p=0.046)	2.25 n.s.

**Table 70. Results of MANOVAs on Morris water maze Trial 1 times and distances averaged over Days 3 through 7.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 5.63 (2,126) p = 0.005	Average Trial 1 Time	9.46 (1,127)	p = 0.003
		Average Trial 1 Distance	4.69 (1,127)	p = 0.032
	<b>SEX</b> 5.33 (2,126) p = 0.006	Average Trial 1 Time	10.57 (1,127)	p = 0.001
		Average Trial 1 Distance	9.67 (1,127)	p = 0.002
	<b>STRESS</b> 9.36 (2,126) p < 0.001	Average Trial 1 Time	1.42 (1,127)	n.s.
		Average Trial 1 Distance	0.56 (1,127)	n.s.
	<b>STRAIN X SEX</b> 1.10 (2,126) n.s.	Average Trial 1 Time	0.16 (1,127)	n.s.
		Average Trial 1 Distance	0.98 (1,127)	n.s.
	<b>STRAIN X STRESS</b> 2.30 (2,126) n.s.	Average Trial 1 Time	4.64 (1,127)	p = 0.033
		Average Trial 1 Distance	3.74 (1,127)	p = 0.055
Sprague-Dawleys	<b>SEX</b> 3.21 (2,66) p = 0.047	Average Trial 1 Time	3.35 (1,127)	p = 0.069
		Average Trial 1 Distance	4.21 (1,127)	p = 0.042
	<b>STRESS</b> 7.67 (2,66) p = 0.001	Average Trial 1 Time	0.00 (1,127)	n.s.
		Average Trial 1 Distance	0.07 (1,127)	n.s.
Long-Evans	<b>SEX</b> 5.12 (2,59) p = 0.009	Average Trial 1 Time	7.21 (1,60)	p = 0.009
		Average Trial 1 Distance	10.38 (1,60)	p = 0.002
	<b>STRESS</b> 4.67 (2,59) p = 0.013	Average Trial 1 Time	6.06 (1,60)	p = 0.017
		Average Trial 1 Distance	0.86 (1,60)	n.s.
SD Males	<b>SEX X STRESS</b> 1.66 (2,59) n.s.	Average Trial 1 Time	1.78 (1,60)	n.s.
		Average Trial 1 Distance	3.31 (1,60)	p = 0.074
	<b>STRESS</b> 4.71 (2,31) p = 0.016	Average Trial 1 Time	0.22 (1,32)	n.s.
		Average Trial 1 Distance	0.19 (1,32)	n.s.
SD Females	<b>STRESS</b> 4.33 (2,34) p = 0.021	Average Trial 1 Time	1.66 (1,35)	n.s.
		Average Trial 1 Distance	4.09 (1,35)	p = 0.051

<b>LE Males</b>	<b>STRESS</b> 3.10 (2,29) <i>p</i> = 0.060	<b>Average Trial 1 Time</b>	<b>6.40 (1,30)</b>	<b><i>p</i> = 0.017</b>
		<b>Average Trial 1 Distance</b>	<b>5.11 (1,30)</b>	<b><i>p</i> = 0.031</b>
<b>LE Females</b>	<b>STRESS</b> 2.31 (2,29) <i>n.s.</i>	<b>Average Trial 1 Time</b>	<b>0.73 (1,30)</b>	<b><i>n.s.</i></b>
		<b>Average Trial 1 Distance</b>	<b>0.31 (1,30)</b>	<b><i>n.s.</i></b>

**Table 71. Results of MANOVAs on Morris water maze Trial 2 times and distances averaged over Days 3 through 7.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 4.00 (2,126) p = 0.021	Average Trial 2 Time	8.05 (1,127)	p = 0.005
		Average Trial 2 Distance	6.77 (1,127)	p = 0.010
	<b>SEX</b> 9.99 (2,126) p < 0.001	Average Trial 2 Time	17.08 (1,127)	p < 0.001
		Average Trial 2 Distance	10.20 (1,127)	p = 0.002
	<b>STRESS</b> 2.23 (2,126) n.s.	Average Trial 2 Time	1.20 (1,127)	n.s.
		Average Trial 2 Distance	0.11 (1,127)	n.s.
	<b>STRAIN X SEX</b> 0.71 (2,126) n.s.	Average Trial 2 Time	0.48 (1,127)	n.s.
		Average Trial 2 Distance	0.08 (1,127)	n.s.
	<b>STRAIN X STRESS</b> 2.13 (2,126) n.s.	Average Trial 2 Time	0.75 (1,127)	n.s.
		Average Trial 2 Distance	0.00 (1,127)	n.s.
Sprague-Dawleys	<b>SEX</b> 4.07 (2,66) p = 0.022	Average Trial 2 Time	6.88 (1,67)	p = 0.011
		Average Trial 2 Distance	4.77 (1,67)	p = 0.033
	<b>STRESS</b> 0.01 (2,66) n.s.	Average Trial 2 Time	0.03 (1,67)	n.s.
		Average Trial 2 Distance	0.03 (1,67)	n.s.
	<b>SEX X STRESS</b> 1.40 (2,66) n.s.	Average Trial 2 Time	0.67 (1,67)	n.s.
Long-Evans		Average Trial 2 Distance	0.14 (1,67)	n.s.
	<b>SEX</b> 5.61 (2,59) p = 0.006	Average Trial 2 Time	10.03 (1,60)	p = 0.002
		Average Trial 2 Distance	5.37 (1,60)	p = 0.024
	<b>STRESS</b> 2.70 (2,59) p = 0.076	Average Trial 2 Time	1.66 (1,60)	n.s.
		Average Trial 2 Distance	0.08 (1,60)	n.s.
	<b>SEX X STRESS</b> 0.11 (2,59) n.s.	Average Trial 2 Time	0.00 (1,60)	n.s.
		Average Trial 2 Distance	0.05 (1,60)	n.s.

**Table 72. Results of MANOVAs on corticosterone and ACTH for animals that were sacrificed on Stress Day 11.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 3.37 (2,23) p = 0.052	Corticosterone	1.07 (1,24)	n.s.
		ACTH	3.45 (1,24)	p = 0.076
	<b>SEX</b> 7.51 (2,23) p = 0.003	Corticosterone	15.58 (1,24)	p = 0.001
		ACTH	1.54 (1,24)	n.s.
	<b>STRESS</b> 11.32 (2,23) p < 0.001	Corticosterone	19.81 (1,24)	p < 0.001
		ACTH	12.35 (1,24)	p = 0.002
	<b>STRAIN X SEX</b> 1.31 (2,23) n.s.	Corticosterone	2.56 (1,24)	n.s.
		ACTH	0.05 (1,24)	n.s.
	<b>STRAIN X STRESS</b> 3.26 (2,23) p = 0.057	Corticosterone	4.65 (1,24)	p = 0.041
		ACTH	4.78 (1,24)	p = 0.039
Males	<b>SEX X STRESS</b> 1.00 (2,23) n.s.	Corticosterone	0.79 (1,24)	n.s.
		ACTH	0.51 (1,24)	n.s.
	<b>STRAIN X SEX X STRESS</b> 0.12 (2,23) n.s.	Corticosterone	0.20 (1,24)	n.s.
		ACTH	0.00 (1,24)	n.s.
Females	<b>STRAIN</b> 1.67 (2,11) n.s.	Corticosterone	0.52 (1,12)	n.s.
		ACTH	3.65 (1,12)	p = 0.080
	<b>STRESS</b> 21.16 (2,11) p < 0.001	Corticosterone	46.16 (1,12)	p < 0.001
		ACTH	6.61 (1,12)	p = 0.025
	<b>STRAIN X STRESS</b> 2.95 (2,11) p = 0.094	Corticosterone	4.74 (1,12)	p = 0.050
		ACTH	4.17 (1,12)	p = 0.064
	<b>STRAIN</b> 2.22 (2,11) n.s.	Corticosterone	2.05 (1,12)	n.s.
		ACTH	0.95 (1,12)	n.s.
SD Males	<b>STRESS</b> 57.05 (2,5) p < 0.001	Corticosterone	3.76 (1,12)	p = 0.077
		ACTH	6.35 (1,12)	p = 0.027
	<b>STRAIN X STRESS</b> 1.20 (2,11) n.s.	Corticosterone	2.00 (1,12)	n.s.
		ACTH	1.64 (1,12)	n.s.
SD Females	<b>STRESS</b> 6.73 (2,5) p = 0.038	Corticosterone	14.79 (1,6)	p = 0.008
		ACTH	4.17 (1,6)	p = 0.087

<b>LE Males</b>	<b>STRESS</b> 3.16 (2,5) n.s.	<b>Corticosterone</b>	<b>6.47 (1,6)</b>	<b>p = 0.044</b>
		<b>ACTH</b>	<b>1.55 (1,6)</b>	<b>n.s.</b>
<b>LE Females</b>	<b>STRESS</b> 4.32 (2,5) p = 0.081	<b>Corticosterone</b>	<b>0.08 (1,6)</b>	<b>n.s.</b>
		<b>ACTH</b>	<b>2.86 (1,6)</b>	<b>n.s.</b>

**Table 73: Results of ANOVAs on CRF from animals sacrificed on Stress Day 11.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Strain	2.87 (1,24)	n.s.
	Sex	8.02 (1,24)	p=0.009
	Stress	0.20 (1,24)	n.s.
	Strain X Sex	53.15 (1,24)	p < 0.001
	Strain X Stress	7.11 (1,24)	p = 0.014
	Sex X Stress	0.01 (1,24)	n.s.
	Strain X Sex X Stress	0.15 (1,24)	n.s.
Males	Strain	49.89 (1,12)	p < 0.001
	Stress	0.19 (1,12)	n.s.
	Strain X Stress	3.20 (1,12)	p = 0.099
Females	Strain	13.15 (1,12)	p = 0.003
	Stress	0.05 (1,12)	n.s.
	Strain X Stress	3.92 (1,12)	p=0.071
SD Males	Stress	0.89 (1,6)	n.s.
SD Females	Stress	4.23 (1,6)	p=0.086
LE Males	Stress	2.54 (1,6)	n.s.
LE Females	Stress	1.49 (1,6)	n.s.

**Table 74. Results of MANOVAs on corticosterone and ACTH for animals that were sacrificed on Stress Day 21.**

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All animals	<b>STRAIN</b> 2.07 (2,71) n.s.	Corticosterone	1.61 (1,72)	n.s.
	ACTH	4.07 (1,72)	p = 0.047	
	<b>SEX</b> 12.43 (2,71) p < 0.001	Corticosterone	18.21 (1,72)	p < 0.001
	ACTH	0.07 (1,72)	n.s.	
	<b>STRESS</b> 34.74 (2,71) p < 0.001	Corticosterone	69.30 (1,72)	p < 0.001
	ACTH	24.50 (1,72)	p < 0.001	
	<b>STRAIN X SEX</b> 5.03 (2,71) p = 0.009	Corticosterone	0.06 (1,72)	n.s.
	ACTH	7.14 (1,72)	p = 0.009	
	<b>STRAIN X STRESS</b> 4.30 (2,71) p = 0.017	Corticosterone	5.40 (1,72)	p = 0.024
	ACTH	7.41 (1,72)	p = 0.008	
Males	<b>SEX X STRESS</b> 0.81 (2,71) n.s.	Corticosterone	0.02 (1,72)	n.s.
	ACTH	1.14 (1,72)	n.s.	
	<b>STRAIN X SEX X STRESS</b> 1.54 (2,71) n.s.	Corticosterone	0.18 (1,72)	n.s.
	ACTH	1.69 (1,72)	n.s.	
Females	<b>STRAIN</b> 13.23 (2,35) p < 0.001	Corticosterone	1.24 (1,36)	n.s.
	ACTH	25.80 (1,36)	p < 0.001	
	<b>STRESS</b> 45.80 (2,35) p < 0.001	Corticosterone	85.51 (1,36)	p < 0.001
	ACTH	44.16 (1,36)	p < 0.001	
	<b>STRAIN X STRESS</b> 9.25 (2,35) p = 0.001	Corticosterone	4.25 (1,36)	p = 0.047
	ACTH	18.99 (1,36)	p < 0.001	
SD Males	<b>STRAIN</b> 0.76 (2,35) n.s.	Corticosterone	0.73 (1,36)	n.s.
	ACTH	0.14 (1,36)	n.s.	
	<b>STRESS</b> 10.31 (2,35) p < 0.001	Corticosterone	21.17 (1,36)	p < 0.001
	ACTH	4.50 (1,36)	p = 0.041	
SD Females	<b>STRAIN X STRESS</b> 1.15 (2,35) n.s.	Corticosterone	2.37 (1,36)	n.s.
	ACTH	0.64 (1,36)	n.s.	
	<b>STRESS</b> 91.84 (2,17) p < 0.001	Corticosterone	192.67 (1,18)	p < 0.001
	ACTH	43.40 (1,18)	p < 0.001	
LE Males	<b>STRESS</b> 16.50 (2,17) p < 0.001	Corticosterone	31.84 (1,18)	p < 0.001
	ACTH	9.80 (1,18)	p = 0.006	
	<b>STRESS</b> 7.36 (2,17) p = 0.005	Corticosterone	15.48 (1,18)	p = 0.001
	ACTH	4.32 (1,18)	p = 0.052	
LE Females	<b>STRESS</b> 1.64 (2,17) n.s.	Corticosterone	3.33 (1,18)	p = 0.085
	ACTH	0.56 (1,18)	n.s.	

**Table 75: Results of ANOVAs on CRF from animals sacrificed on Stress Day 21.**

<b>Group Tested</b>	<b>Effect</b>	<b>F value (d.f.)</b>	<b>p value</b>
All animals	Strain	10.88 (1,68)	p = 0.002
	Sex	27.31 (1,68)	p < 0.001
	Stress	1.63 (1,68)	n.s.
	Strain X Sex	21.98 (1,68)	p < 0.001
	Strain X Stress	0.01 (1,68)	n.s.
	Sex X Stress	1.02 (1,68)	n.s.
	Strain X Sex X Stress	0.25 (1,68)	n.s.
Males	Strain	2.18 (1,36)	n.s.
	Stress	0.08 (1,36)	n.s.
	Strain X Stress	0.18 (1,36)	n.s.
Females	Strain	18.91 (1,36)	p < 0.001
	Stress	1.55 (1,36)	n.s.
	Strain X Stress	0.11 (1,36)	n.s.
SD Males	Stress	0.18 (1,18)	n.s.
SD Females	Stress	0.02 (1,18)	n.s.
LE Males	Stress	0.56 (1,18)	n.s.
LE Females	Stress	0.98 (1,18)	n.s.

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